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(54) FRAMEWORK MUTATED ANTIBODIES AND THEIR PREPARATION

RAHMENBAU-MUTIERTE ANTIKÖRPER UND IHRE HERSTELLUNG

ANTICORPS A MUTATION DE SQUELETTE ET LEUR PREPARATION

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Description

The present invention relates to altered antibodies and their preparation. The invention is typically applicable to the production of humanised antibodies.

Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs are held in close proximity by the framework regions and, with the CDRs from the other domain, contribute to the formation of the antigen binding site.

The preparation of an altered antibody in which the CDRs are derived from a different species than the framework of the antibody's variable domains is disclosed in EP-A-0239400. The CDRs may be derived from a rat or mouse monoclonal antibody. The framework of the variable domains, and the constant domains, of the altered antibody may be derived from a human antibody. Such a humanised antibody elicits a negligible immune response when administered to a human compared to the immune response mounted by a human against a rat or mouse antibody. Humanised CAMPATH-1 antibody is disclosed in EP-A-0328404.

WO-A-90 07861 relates to a humanised antibody specific for the p55 Tac protein of the IL-2 receptor in which the amino acid sequence of the CDRs is that of a mouse antibody against the same antigen and the amino acid sequence of the variable domain framework regions is that of a human antibody chosen on the basis of homology with the framework regions of the rodent antibody. The DNA encoding the humanised antibody is produced by conventional methods, for example use of synthetic oligonucleotides.

We have now devised a new way of preparing an altered antibody. In contrast to previous proposals, this involves altering the framework of a variable domain rather than the CDRs. This approach has the advantages that it does not require a pre-existing cDNA encoding, for example, a human framework to which to reshape and that it is technically easier than prior methodologies.

Accordingly, the present invention provides a process for the preparation of an antibody chain in which the CDRs of the variable domain of the antibody chain are derived from a first mammalian species and the framework of the variable domain and, if present, the or each constant domain of the antibody chain are derived from a second different mammalian species, which process comprises:

(i) mutating the framework-encoding regions of DNA encoding a variable domain of an antibody chain of the said first species such that the mutated framework-encoding regions encode the said framework derived from the said second species; and

(ii) expressing the said antibody chain utilising the mutated DNA from step (i);

the mutation in step (i) being such that an antibody incorporating the antibody chain expressed in step (ii) retains the binding capability of the antibody from which the CDRs are derived.

A variable domain of either or both chains of an antibody can therefore be altered by:

(a) determining the nucleotide and predicted amino acid sequence of a variable domain of a selected antibody chain of the said first species;

(b) determining the antibody framework to which the framework of the said variable domain is to be altered;

(c) mutating the framework-encoding regions of DNA encoding the said variable domain such that the mutated framework-encoding regions encode the framework determined upon in step (b);

(d) linking the mutated DNA obtained in step (c) to DNA encoding a constant domain of the said second species and cloning the DNA into an expression vector; and

(e) introducing the expression vector into a compatible host cell and culturing the host cell under such conditions that antibody chain is expressed.

The antibody chain may be co-expressed with a complementary antibody chain. At least the framework of the variable domain and the or each constant domain of the complementary chain generally are derived from the said second species also. A light chain and a heavy chain may be co-expressed. Either or both chains may have been

prepared by the process of the invention. Preferably the CDRs of both chains are derived from the same selected antibody. An antibody comprising both expressed chains can be recovered.

The antibody preferably has the structure of a natural antibody or a fragment thereof. The antibody may therefore comprise a complete antibody, a $(Fab')_2$ fragment, a Fab fragment, a light chain dimer or a heavy chain. The antibody may be an IgG such as an IgG1, IgG2, IgG3 or IgG4 IgM, IgA, IgE or IgD. Alternatively, the antibody may be a chimaeric antibody of the type described in WO 86/01533.

A chimaeric antibody according to WO 86/01533 comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain or heavy chain variable domain. Typically, the chimaeric antibody comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused at its C-terminus to the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme region, a region derived from a protein having known binding specificity, from a protein toxin or indeed from any protein expressed by a gene. The two regions of the chimaeric antibody may be connected via a cleavable linker sequence.

The invention is preferably employed to humanise an antibody, typically a monoclonal antibody and, for example, a rat or mouse antibody. The framework and constant domains of the resulting antibody are therefore human framework and constant domains whilst the CDRs of the light and/or heavy chain of the antibody are rat or mouse CDRs. Preferably all CDRs are rat or mouse CDRs. The antibody may be a human IgG such as IgG1, IgG2, IgG3, IgG4; IgM; IgA; IgE or IgD carrying rat or mouse CDRs.

The process of the invention is carried out in such a way that the resulting antibody retains the antigen binding capability of the antibody from which it is derived. An antibody is reshaped according to the invention by mutating the framework-encoding regions of DNA coding for the variable domains of the antibody. This antibody and the reshaped antibody should both be capable of binding to the same antigen.

The starting antibody is typically an antibody of a selected specificity. In order to ensure that this specificity is retained, the variable domain framework of the antibody is preferably reshaped to about the closest variable domain framework of an antibody of another species. By "about the closest" is meant about the most homologous in terms of amino acid sequences. Preferably there is a homology of at least 50% between the two variable domains.

There are four general steps to reshape a monoclonal antibody. These are:

(1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy chain variable domains;
(2) designing the reshaped antibody, i.e. deciding which antibody framework region to use during the reshaping process;
(3) the actual reshaping methodologies/techniques; and
(4) the transfection and expression of the reshaped antibody.

These four steps are explained below in the context of humanising an antibody. However, they may equally well be applied when reshaping to an antibody of a non-human species.

Step 1: Determining the nucleotide and predicted amino acid sequence of the antibody light and heavy chain variable domains

To reshape an antibody only the amino acid sequence of antibody's heavy and light chain variable domains needs to be known. The sequence of the constant domains is irrelevant because these do not contribute to the reshaping strategy. The simplest method of determining an antibody's variable domain amino acid sequence is from cloned cDNA encoding the heavy and light chain variable domain.

There are two general methods for cloning a given antibody's heavy and light chain variable domain cDNAs: (1) via a conventional cDNA library, or (2) via the polymerase chain reaction (PCR). Both of these methods are widely known. Given the nucleotide sequence of the cDNAs, it is a simple matter to translate this information into the predicted amino acid sequence of the antibody variable domains.

Step 2: Designing the reshaped antibody

There are several factors to consider in deciding which human antibody sequence to use during the reshaping. The reshaping of light and heavy chains are considered independently of one another, but the reasoning is basically similar for each.

This selection process is based on the following rationale: A given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable domain framework residues have little or no direct contribution. The primary function of the framework regions is to hold the CDRs in their proper

spacial orientation to recognize antigen. Thus the substitution of rodent CDRs into a human variable domain framework is most likely to result in retention of their correct spacial orientation if the human variable domain is highly homologous to the rodent variable domain from which they originated. A human variable domain should preferably be chosen therefore that is highly homologous to the rodent variable domain(s).

A suitable human antibody variable domain sequence can be selected as follows:

1. Using a computer program, search all available protein (and DNA) databases for those human antibody variable domain sequences that are most homologous to the rodent antibody variable domains. This can be easily accomplished with a program called FASTA but other suitable programs are available. The output of a suitable program is a list of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each sequence to the rodent sequence. This is done independently for both the heavy and light chain variable domain sequences. The above analyses are more easily accomplished if customized sub-databases are first created that only include human immunoglobulin sequences. This has two benefits. First, the actual computational time is greatly reduced because analyses are restricted to only those sequences of interest rather than all the sequences in the databases. The second benefit is that, by restricting analyses to only human immunoglobulin sequences, the output will not be cluttered by the presence of rodent immunoglobulin sequences. There are far more rodent immunoglobulin sequences in databases than there are human.
2. List the human antibody variable domain sequences that have the most overall homology to the rodent antibody variable domain (from above). Do not make a distinction between homology within the framework regions and CDRs. Consider the overall homology.
3. Eliminate from consideration those human sequences that have CDRs that are a different length than those of the rodent CDRs. This rule does not apply to CDR 3, because the length of this CDR is normally quite variable. Also, there are sometimes no or very few human sequences that have the same CDR lengths as that of the rodent antibody. If this is the case, this rule can be loosened, and human sequences with one or more differences in CDR length can be allowed.
4. From the remaining human variable domains, the one is selected that is most homologous to that of the rodent.
5. The actual reshaped antibody (the end result) should contain CDRs derived from the rodent antibody and a variable domain framework from the human antibody chosen above.

Step 3: The actual reshaping methodologies/techniques

A cDNA encoding the desired reshaped antibody is preferably made beginning with the rodent cDNA from which the rodent antibody variable domain sequence(s) was originally determined. The rodent variable domain amino acid sequence is compared to that of the chosen human antibody variable domain sequence. The residues in the rodent variable domain framework are marked that need to be changed to the corresponding residue in the human to make the rodent framework identical to that of the human framework. There may also be residues that need adding to or deleting from the rodent framework sequence to make it identical to that of the human.

Oligonucleotides are synthesised that can be used to mutagenize the rodent variable domain framework to contain the desired residues. Those oligonucleotides can be of any convenient size. One is normally only limited in length by the capabilities of the particular synthesizer one has available. The method of oligonucleotide-directed in vitro mutagenesis is well known.

The advantages of this method of reshaping as opposed to splicing CDRs into a human framework are that (1) this method does not require a pre-existing cDNA encoding the human framework to which to reshape and (2) splicing CDRs is technically more difficult because there is usually a large region of poor homology between the mutagenic oligonucleotide and the human antibody variable domain. This is not so much a problem with the method of splicing human framework residues onto a rodent variable domain because there is no need for a pre-existing cDNA encoding the human variable domain. The method starts instead with the rodent cDNA sequence. Also, splicing framework regions is technically easier because there is a high degree of homology between the mutagenic oligonucleotide and the rodent variable domain framework. This is true because a human antibody variable domain framework has been selected that is most homologous to that of the rodent.

The advantage of the present method of reshaping as opposed to synthesizing the entire reshaped version from scratch is that it is technically easier. Synthesizing a reshaped variable domain from scratch requires several more oligonucleotides, several days more work, and technical difficulties are more likely to arise.

Step 4: The transfection and expression of the reshaped antibody

Following the mutagenesis reactions to reshape the antibody, the cDNAs are linked to the appropriate DNA encoding light or heavy chain constant region, cloned into an expression vector, and transfected into mammalian cells.

These steps can be carried out in routine fashion. A reshaped antibody may therefore be prepared by a process comprising:

a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a first antibody and CDRs comprising at least parts of the CDRs from a second antibody of different specificity;
b) if necessary, preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;
c) transforming a cell line with the first or both prepared vectors; and
d) culturing said transformed cell line to produce said altered antibody.

Preferably the DNA sequence in step a) encodes both the variable domain and the or each constant domain of the antibody chain, the or each constant domain being derived from the first antibody. The antibody can be recovered and purified. The cell line which is transformed to produce the altered antibody may be a Chinese Hamster Ovary (CHO) cell line or an immortalised mammalian cell line, which is advantageously of lymphoid origin, such as a myeloma, hybridoma, trioma or quadroma cell line. The cell line may also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof.

Although the cell line used to produce the altered antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a yeast cell line, may alternatively be used. In particular, it is envisaged that E. coli - derived bacterial strains could be used.

It is known that some immortalised lymphoid cell lines, such as myeloma cell lines, in their normal state secrete isolated Ig light or heavy chains. If such a cell line is transformed with the vector prepared in step (a) it will not be necessary to carry out step (b) of the process, provided that the normally secreted chain is complementary to the variable domain of the Ig chain encoded by the vector prepared in step (a).

However, where the immortalised cell line does not secrete or does not secrete a complementary chain, it will be necessary to carry out step (b). This step may be carried out by further manipulating the vector produced in step (a) so that this vector encodes not only the variable domain of an altered antibody light or heavy chain, but also the complementary variable domain.

Alternatively, step (b) is carried out by preparing a second vector which is used to transform the immortalised cell line. This alternative leads to easier construct preparation, but may be less preferred than the first alternative in that it may not lead to as efficient production of antibody.

In the case where the immortalised cell line secretes a complementary light or heavy chain, the transformed cell line may be produced for example by transforming a suitable bacterial cell with the vector and then fusing the bacterial cell with the immortalised cell line by spheroplast fusion. Alternatively, the DNA may be directly introduced into the immortalised cell line by electroporation or other suitable method.

An antibody is consequently produced in which CDRs of a variable domain of an antibody chain are homologous with the corresponding CDRs of an antibody of a first mammalian species and in which the framework of the variable domain and the constant domains of the antibody are homologous with the corresponding framework and constant domains of an antibody of a second, different, mammalian species. Typically, all three CDRs of the variable domain of a light or heavy chain are derived from the first species.

The present process has been applied to obtain an antibody against human CD4 antigen. Accordingly, the invention also provides an antibody which is capable of binding to human CD4 antigen, in which the CDRs of the light chain of the antibody have the amino acid sequences:

CDR1: LASEDIYSDLA
CDR2: NTDTLQN
CDR3: QQYNNYPWT,

in which the CDRs of the heavy chain of the antibody have the amino acid sequences:

CDR1: NYGMA
CDR2: TISHDGSDTYFRDSVKG
CDR3: QGTIAGIRH, and

in which the framework of the variable domain and, if present, the or each constant domain of each chain are derived

from a mammalian non-rat species.

The antibody preferably has the structure of a natural antibody or a fragment thereof. The antibody may therefore comprise a complete antibody, a $(Fab')_2$ fragment, a Fab fragment, a light chain dimer or a heavy chain.

The antibody may be an IgG such as IgG1, IgG2, IgG3 or IgG4 IgM, IgA, IgE or IgD. Alternatively, the antibody may be a chimaeric antibody of the type described in WO 86/01533.

A chimaeric antibody according to WO 86/01533 comprises an antigen binding region and a non-immunoglobulin region. The antigen binding region is an antibody light chain variable domain or heavy chain variable domain. Typically the chimaeric antibody comprises both light and heavy chain variable domains. The non-immunoglobulin region is fused at its C-terminus to the antigen binding region. The non-immunoglobulin region is typically a non-immunoglobulin protein and may be an enzyme region, a region derived from a protein having known binding specificity, from a protein toxin or indeed from any protein expressed by a gene. The two regions of the chimaeric antibody may be connected via a cleavable linker sequence.

The invention is preferably employed to humanise a CD4 antibody such as a rat or mouse CD4 antibody. The framework and the constant domains of the resulting antibody are therefore human framework and constant domains whilst the CDRs of the light and/or heavy chain of the antibody are rat or mouse CDRs. Preferably all CDRs are rat or mouse CDRs. The antibody may be a human IgG such as IgG1, IgG2, IgG3, IgG4; IgM; IgA; IgE or IgD carrying rat or mouse CDRs.

Preferably the framework of the antibody heavy chain is homologous to the corresponding framework of the human antibody KOL (schmidt et al, Hoppe-Seyler's Z. Physiol. Chem., 364 713-747, 1983). The sixth residue of framework 4 in this case is suitably Thr or Pro, preferably Thr. This residue is the 121st residue in the KOL antibody heavy chain variable region (Schmidt et al, 1983), and is identified as residue 108 by Kabat (Kabat et al, "Sequences of proteins of immunological interest", US Dept of Health and Human Services, US Government Printing Office, 1987). Alternatively, the framework of the antibody heavy chain is homologous to the corresponding framework of the human antibody NEW (Saul et al, J. Biol.Chem. 253: 585-597, 1978). The final residue of framework 1 in this case is suitably Ser or Thr, preferably Ser. This residue is at position 30 (Kabat et al, 1987). Preferably the framework of the antibody light chain is homologous to the variable domain framework of the protein REI (Epp et al, Eur. J. Biochem., 45, 513-524, 1974).

The framework regions of one or both chains of a CD4 antibody can be reshaped by the present process. Alternatively, one or both chains of a CD4 antibody may be reshaped by the procedure described in EP-A-0239400. The procedure of EP-A-0239400 involves replacing CDRs rather than the replacement of frameworks. The CDRs are grafted onto a framework derived from a mammalian non-rat species, typically a human. This may be achieved by oligonucleotide-directed in vitro mutagenesis of the CDR-encoding regions of an antibody chain, light or heavy, from a mammalian non-rat species. The oligonucleotides in such an instance are selected so that the resulting CDR-grafted antibody has the light chain CDRs 1 to 3 and the heavy chain CDRs 1 to 3 shown above.

The reshaped CD4 antibody can be used to induce tolerance to an antigen. It can be used to alleviate autoimmune diseases such as rheumatoid arthritis. It can be used to prevent graft rejection. Tolerance to a graft such as an organ graft or a bone marrow transplantation can be achieved. Also, the reshaped CD4 antibody might be used to alleviate allergies. Tolerance to allergens could be achieved.

The CD4 antibody may be depleting or non-depleting. A depleting antibody is an antibody which depletes more than 50%, for example from 90 to 99%, of target cells in vivo. A non-depleting antibody depletes fewer than 50%, for example, from 10 to 25% and preferably less than 10% of target cells in vivo. A CD4 antibody may be administered alone or may be co-administered with a non-depleting or depleting CD8 antibody. The CD4 antibody, depleting or non-depleting, and CD8 monoclonal antibody, depleting or non-depleting, may be administered sequentially in any order or may be administered simultaneously. An additional antibody, drug or protein may be administered before, during or after administration of the antibodies.

A CD4 antibody and, indeed, a CD8 antibody as appropriate are given parenterally, for example intravenously. The antibody may be administered by injection or by infusion. For this purpose the antibody is formulated in a pharmaceutical composition further comprising a pharmaceutically acceptable carrier or diluent. Any appropriate carrier or diluent may be employed, for example phosphate-buffered saline solution.

The amount of non-depleting or depleting CD4 and, if desired, CD8 antibody administered to a patient depends upon a variety of factors including the age and weight of a patient, the condition which is being treated and the antigen (s) to which it is desired to induce tolerance. In a model mouse system from 1μg to 2mg, preferably from 400μg to lmg, of a mAb is administered at any one time. In humans from 3 to 500mg, for example from 5 to 200mg, of antibody may be administered at any one time. Many such doses may be given over a period of several weeks, typically 3 weeks.

A foreign antigen(s) to which it is desired to induce tolerance can be administered to a host before, during, or after a course of CD4 antibody (depleting or non-depleting) and/or CD8 antibody (depleting or non-depleting). Typically, however, the antigen(s) is administered one week after commencement of antibody administration, and is terminated three weeks before the last antibody administration.

Tolerance can therefore be induced to an antigen in a host by administering non-depleting or depleting CD4 and CD8 mAbs and, under cover of the mAbs, the antigen. A patient may be operated on surgically under cover of the non-depleting or depleting CD4 and CD8 mAbs to be given a tissue transplant such as an organ graft or a bone marrow transplant. Also, tolerance may be induced to an antigen already possessed by a subject. Long term specific tolerance can be induced to a self antigen or antigens in order to treat autoimmune disease such as multiple sclerosis or rheumatoid arthritis. The condition of a patient suffering from autoimmune disease can therefore be alleviated.

The following Example illustrates the invention. In the accompanying drawings:

Figure 1: shows the nucleotide and predicted amino acid sequence of rat CD4 antibody light chain variable region. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. Base pairs 1-269 (HindIII-PvuII) and 577-620 ([Bg1II/Bc1I]-BamHI) are part of the vector M13V_KPCR3, while base pairs 270-576 are from the PCR product of the CD4 antibody light chain variable region (V_L). CDRs (boxes) were identified by comparison to known immunological sequences (Kabat et al, "Sequences of proteins of immunological interest, US Dept of Health and Human Services, US Government Printing Office, 1987).

Figure 2: shows the nucleotide and predicted amino acid sequence of the reshaped CAMPATH-1 antibody light chain cDNA. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 3: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody light chain cDNA CD4V_LREI. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 4: shows the nucleotide and predicted amino acid sequence of rat CD4 antibody heavy chain variable region. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes. Base pairs 1-272 (HindIII-PstI) and 603-817 (BstEII-BamHI) are part of the vector M13V_HPCR1, while base pairs 273-602 are from the PCR product of the CD4 antibody heavy chain variable region (V_H).

Figure 5: shows the nucleotide and predicted amino acid sequence of the reshaped CAMPATH-1 antibody heavy chain cDNA. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 6: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain cDNA CD4V_HNEW-Thr^{30}. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 7: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain cDNA CD4V_HNEW-Ser^{30}. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 8: shows the heavy chain variable (V) region amino acid sequence of the human myeloma protein KOL. CDRs are identified by boxes. This sequence is taken from the Swiss-Prot protein sequence database.

Figure 9: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V_HKOL-Pro^{113}. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 10: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V_HKOL-Pro^{113} without immunoglobulin promoter. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 11: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V_HKOL-Thr^{113}. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 12: shows the nucleotide and predicted amino acid sequence of the reshaped CD4 antibody heavy chain V region CD4V_HKOL-Thr^{113} without immunoglobulin promoter. The number of the first and last amino acid or nucleotide in each line is indicated in the left and right margins, respectively. CDRs are identified by boxes.

Figure 13: shows the results of an ELISA that compares the avidity of YNB46.1.8 and CD4V_HKOL-Thr^{113} antibodies. The X-axis indicates the concentration (μg/ml) of YNB46.1.8 (triangles) or CD4V_HKOL-Thr^{113} (circles) antibody. The Y-axis indicates the optical density at 492 nanometers.

EXAMPLE

1. MATERIALS AND METHODS

Isolation of monoclonal antibody. The rat-derived anti-human CD4 antibody, clone YNB46.1.8 (IgG_{2b}, kappa light chain serotype), was the result of fusion between a rat splenocyte and the Lou strain rat myeloma cell line Y3-Ag 1.2.3 (Galfre et al, Nature, 277: 131-133, 1979) and was selected by its binding to a rat T cell line NB2-6TG stably transfected

with an expression vector containing a complementary DNA (cDNA) encoding the human CD4 antigen (Madden et al, Cell, 42: 93-104, 1985). Antibody was purified by high pressure liquid chromatography (HPLC).

Isolation of Antibody Variable Regions. cDNAs encoding the V_L and V_H regions of the CD4 antibody were isolated by a polymerase chain reaction (PCR)-based method (Orlandi et al, PNAS USA, 86: 3833-3837, 1989) with some modifications. Total RNA was isolated from hybridoma cells by the guanidine thiocyanate method (Chirgwin et al, Biochemistry, 18: 5294, 1979), and poly(A)$^+$ RNA was isolated by passage of total RNA through and elution from an oligo(dT)-cellulose column (Aviv and Leder PNAS USA 69: 1408, 1972). Poly(A)$^+$ RNA was heated at 70°C for 5 minutes and cooled on ice just prior to use. A 25µl first strand synthesis reaction consisted of 5µg poly(A)$^+$ RNA, 250 µM each dNTP, 50 mM Tris.HC1 (pH 8.2 at 42°C), 10 mM $MgCl_2$, 100 mM KC1, 10 mM dithiothreitol, 23 units reverse transcriptase (Anglian Biotec, Colchester, U.K.), 3.5 pmoles of the V_L region-specific oligonucleotide primer V_K1FOR [5'-d(GTT AGA TCT CCA GCT TGG TCC C)] or the V_H region-specific primer V_H1FOR-B [5,-d(TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC)], and incubated for 5 minutes at 20°C and then 90 minutes at 42°C.

Subsequent 50 µl PCR amplifications consisted of 5 µl of the first strand synthesis reaction (unpurified), 500 µM each dNTP, 67 mM Tris-HC1 (pH 8.8 at 25°C), 17 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$, 20 µg/ml gelatin, 5 units TAQ DNA polymerase (Koch-Light, Haverhill, U.K.), and 25 pmoles (each) of primers V_K1FOR and V_K1BACK [5'-d(GAC ATT CAG CTG ACC CAG TCT CGA)] for the V_L region or V_H1FOR-B and the mixed primer V_H1BACK [5'-d(AG GT(CG) (CA)A(GA) CTG CAG (GC)AG TC(TA) GG)] for the V_H region. Reactions were overlayed with mineral oil and subjected to 30 cycles of 1.5 minutes at 95°C (denaturation), 1.5 minutes at 37°C (V_L) or 50°C (V_H; annealing), and 3 minutes at 72°C (extension) with a Techne PHC-1 programmable cyclic reactor. The final cycle contained a 10 minute extension time.

The samples were frozen at -20°C and the mineral oil (a viscous liquid at -20°C) was removed by aspiration. The aqueous phases were thawed, and PCR products were purified by electrophoresis in 2% agarose gels, and then double digested with either PvuII and BglII (V_L) or PstI and BstEII (V_H) restriction enzymes, and cloned into the PvuII and BclI restriction sites of the vector M13V_KPCR3 (for V_L region; Orlandi et al, 1989) or the PstI and BstEII restriction sites of the vector M13V_HPCR1 (for V_H region). As described in the results, V_L region clones were first screened by hybridisation to a ^{32}P-labeled oligonucleotide probe [5'-d(GTT TCA TAA TAT TGG AGA CA)] specific for the CDR2 of the Y3-Ag 1.2.3 V_L region. V_L region clones not hybridising to this probe and V_H region clones were sequenced by the dideoxy chain termination method (Sanger et al, PNAS USA 74: 5463, 1977).

Reshaped Light Chain Variable Region and Expression Vector Construct.

The reshaped light chain was constructed by oligonucleotide-directed in vitro mutagenesis in an M13 vector by priming with three oligonucleotides simultaneously on a 748 base single-stranded cDNA template encoding the entire V_L and kappa constant (C_K) regions of the reshaped CAMPATH-1 antibody (Reichmann et al, Nature 332: 323-327, 1988). The three oligonucleotides [5'-d(AGA GTG ACC ATC ACC TGT CTA GCA AGT GAG GAC ATT TAC AGT GAT TTA GCA TGG TAC CAG CAG AAG CCA), 5'-d(CTG CTG ATC TAC AAT ACA GAT ACC TTG CAA AAT GGT GTG CCA AGC AGA TTC), 5'-d(ATC GCC ACC TAC TAC TGC CAA CAG TAT AAC AAT TAT CCG TGG ACG TTC GGC CAA GGG ACC)] were designed to replace each of the three CDRs in the REI-based human antibody V_L region framework that is part of the reshaped CAMPATH-1 antibody V_L region (Reichmann et al, 1988). A clone containing each of the three mutant oligonucleotides was identified by nucleotide sequencing and was subcloned into the HindIII site of the expression vector pHβAPr-1 (Gunning et al, PNAS, 84: 4831-4835, 1987) which also contained a dihydrofolate reductase gene (Ringold et al, J.Mol.Appl. Genet. 1: 165-175, 1981) driven by a truncated SV40 promoter.

Reshaped Heavy Chain Variable Regions Based on the Variable Region Framework of the Human Antibody NEW, and Expression Vector Constructs.

Two versions of the NEW-based reshaped heavy chain were created, CD4V_HNEW-Thr^{30} and CD4V_HNEW-Ser^{30}. The CD4V_HNEW-Thr^{30} version (Figure 6) encodes a threonine residue at position 30 while the CD4V_HNEW-Ser^{30} version (Figure 7) encodes a Ser residue at position 30. As a matter of convenience, CD4V_HNEW-Thr^{30} was created first by oligonucleotide-directed in vitro mutagenesis in the vector M13mp18 by priming with three oligonucleotides simultaneously on a 1467 base single-stranded cDNA template (Figure 5) encoding the entire heavy chain of the reshaped CAMPATH-1 antibody (Reichmann et al, 1988). The three oligonucleotides [5'-d(TCT GGC TTC ACC TTC ACC AAC TAT GGC ATG GCC TGG GTG AGA CAG CCA CCT), 5'-d(GGT CTT GAG TGG ATT GGA ACC ATT AGT CAT GAT GGT AGT GAC ACT TAC TTT CGA GAC TCT GTG AAG GGG AGA GTG),5'-d(GTC TAT TAT TGT GCA AGA CAA GGC ACT ATA GCT GGT ATA CGT CAC TGG GGT CAA GGC AGC CTC)] were designed to replace each of the three complementarity determining regions (CDRs) in the NEW-based V_H region that is part of the reshaped CAMPATH-1 antibody (Reichmann et al, 1988). A clone (Figure 6) containing each of the three mutant oligonucleotides was identified by nucleotide sequencing. CD4V_HNEW-Ser^{30} was created second by oligonucleotide-directed in vitro

mutagenesis in the vector M13mp18 by priming with a single oligonucleotide on the 1458 base single-stranded cDNA template (Figure 6) encoding $CD4V_HNEW\text{-}Thr^{30}$. The oligonucleotide [5'-d(GCT TCA CCT TCA GCA ACT ATG GCA T)] was designed to mutate the residue at position 30 from threonine [ACC] to serine [AGC]. A clone (Figure 7) containing this mutant oligonucleotide was identified by nucleotide sequencing. Double-stranded forms of the clones $CD4V_HNEW\text{-}Thr^{30}$ and $CD4V_HNEW\text{-}Ser^{30}$ were subcloned as HindIII fragments into the HindIII site of the expression vector pNH316. The vector pNH316 is a modified version of the vector pHβAPr-1 (Gunning et al, PNAS, 84: 4831-4835, 1987) which was engineered to contain a neomycin resistance gene driven by a metallothionine promoter.

Reshaped Heavy Chain Variable Regions Based on the Variable Region Framework of the Human Antibody KOL, and Expression Vector Constructs

Two versions of the KOL-based reshaped heavy chain were created, $CD4V_HKOL\text{-}Thr^{113}$ and $CD4V_HKOL\text{-}HKO^{113}$. The $CD4V_HKOL\text{-}Thr^{113}$ version encodes a threonine residue at position 113 (Figure 11) while the $CD4V_HKOL\text{-}Pro^{113}$ version encodes a proline residue at position 113 (Figure 9). As a matter of convenience, $CD4V_HKOL\text{-}Thr^{113}$ was created first by oligonucleotide-directed in vitro mutagenesis of single-stranded DNA template containing the 817 base HindIII-BamHI fragment encoding the V_H region of the rat CD4 antibody (Figure 4) cloned into M13mp18 by priming simultaneously with five oligonucleotides [5'-d(CAC TCC CAG GTC CAA CTG GTG GAG TCT GGT GGA GGC GTG GTG CAG CCT GG), 5'-d(AAG GTC CCT GAG ACT CTC CTG TTC CTC CTC TGG ATT CAT CTT CAG TAA CTA TGG CAT G), 5'-d(GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG), 5'-d(ACT ATC TCC AGA GAT AAT AGC AAA AAC ACC CTA TTC CTG CAA ATG G), 5'-d(ACA GTC TGA GGC CCG AGG ACA CGG GCG TGT ATT TCT GTG CAA GAC AAG GGA C)] which were designed to replace the rat framework regions with the human framework regions of KOL. A clone containing each of the five mutant oligonucleotides was identified by nucleotide sequencing. $CD4V_HKOL\text{-}Pro^{113}$ was created second by oligonucleotide-directed in vitro mutagenesis of single-stranded DNA template containing the 817 base HindIII-BamHI fragment encoding $CD4V_HKOL\text{-}Thr^{113}$ cloned into M13mp18 by priming with the oligonucleotide [5'-d(TGG GGC CAA GGG ACC CCC GTC ACC GTC TCC TCA)]. A clone containing this mutant oligonucleotide was identified by nucleotide sequencing.

The immunoglobulin promoters were removed from the double-stranded DNA forms of clones encoding $CD4V_HKOL\text{-}Thr^{113}$ (Figure 11) and $CD4V_HKOL\text{-}Pro^{113}$ (Figure 9) by replacing (for both versions) the first 125 bp (HindIII-NcoI) with a HindIII-NcoI oligonucleotide linker fragment [5'-d(AGC TTT ACA GTT ACT GAG CAC ACA GGA CCT CAC) and its overlapping complement 5'-d(CAT GGT GAG GTC CTG TGT GCT CAG TAA CTG TAA)]. The resultant clones, $CD4V_HKOL\text{-}Thr^{113}$ (Figure 12) and $CD4V_HKOL\text{-}Pro^{113}$ (Figure 10), now 731 bp HindIII-BamHI fragments, were separately subcloned into the HindIII and BamHI cloning sites of the expression vector pHβAPr-1-gpt (Gunning et al, PNAS USA 76, 1373, 1987) into which had been cloned the human IgGI constant region gene (Bruggemann et al, J. Exp.Med. 166, 1351-1361, 1987) at the BamHI site. Thus, when transfected and expressed as antibody heavy chains (see below), these reshaped V_H regions are linked to human IgG1 constant regions.

Fluorescence activated cell sorter (FACS) analysis

The relative affinities of the reshaped antibodies to bind the CD4 antigen were estimated by FACS analysis. The CD4-expressing cells used in this analysis were a cloned rat T cell line NB2-6TG stably transfected with an expression vector containing a complementary DNA (cDNA) encoding the human CD4 antigen (Maddon et al, Cell, 42, 93-104, 1985). Cells were stained with the appropriate reshaped antibody followed by fluorescein-conjugated sheep anti-human antibodies (Binding Site Ltd., Birmingham, UK). Control staining (see Table 1) consisted of no antibody present during the first stage of cell staining. Mean cellular fluorescence was determined with an Ortho FACS.

Antibody avidity analysis

The relative avidities of the rat YNB46.1.8 antibody and the reshaped $CD4V_HKOL\text{-}Thr^{113}$ antibody were estimated by an enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with soluble recombinant CD4 antigen (Byrn et al, Nature, 344: 667-670, 1990) at 50 ul/well, 10 ug/ml, and then blocked with 100 ul/well phosphate buffered saline (PBS) containing 1.0% bovine serum albumin (BSA). Antibodies were diluted in PBS containing 0.1% BSA, and added to wells (50 ul/well) for 45 minutes at room temperature. Biotinylated $CD4V_HKOL\text{-}Thr^{113}$ antibody (10 ul/well; 20 ug/ml final concentration) was then added to each well for an additional 45 minutes. Wells were washed with PBS containing 0.1% BSA, and then 50 ul streptavidin-biotinylated horseradish peroxidase complex (Amersham; Aylesbury, UK) diluted 1:1,000 was added to each well for 30 minutes. Wells were washed with PBS containing 0.1% BSA, and 100 ul substrate (25 mM citric acid, 50 mM disodium hydrogen phosphate, 0.1% (w/v) o-phenylene diamine, 0.04% (v/v) 30% hydrogen peroxide) was added to each well. Reactions were stopped by the addition of 50 ul/well 1.0 M sulfuric acid. Optical densities at 492 nanometers (OD_{492}) were determined with an ELISA plate reader.

Transfections.

Dihydrofolate reductase deficient chinese hamster ovary (CHO^{DHFR-}) cells (10^6/T-75 flask) were cotransfected as described (Wigler et al, PNAS USA 76, 1373, 1979) with 9μg of heavy chain construct and 1 μg of the light chain construct. Transfectants were selected in medium containing 5% dialysed foetal bovine serum for 2 to 3 weeks, and antibody-secreting clones were identified by ELISAs of conditioned media. Antibody was concentrated and purified by protein-A Sepharose (Trade Mark) column chromatography.

2. RESULTS

Cloning of Light and Heavy Chain Variable Region cDNAs.

cDNAs encoding the V_L and V_H regions from CD4 antibody-secreting hybridoma cells were isolated by PCR using primers which amplify the segment of mRNA encoding the N-terminal region through to the J region (Orlandi et al, 1989). V_L and V_H region PCR products were subcloned into the M13-based vectors $M13V_KPCR3$ and $M13V_HPCR1$, respectively. Initial nucleotide sequence analysis of random V_L region clones revealed that most of the cDNAs encoded the V_L region of the light chain expressed by the Y3-Ag 1.2.3 rat myeloma cell line (Crowe et al, Nucleic Acid Research, 17: 7992, 1989) that was used as the fusion partner to generate the anti-CD4 hybridoma. It is likely that the expression of the Y3-Ag 1.2.3 light chain mRNA is greater than that of the CD4 antibody light chain, or the Y3-Ag 1.2.3 light chain mRNA is preferentially amplified during the PCR.

To maximize the chance of finding CD4 V_L region cDNAs, we first screened all M13 clones by hybridisation to a ^{32}P-labeled oligonucleotide probe that is complementary to the CDR 2 of Y3-Ag 1.2.3 (Crowe et al, Nucleic Acid Research, 17: 7992, 1989). Subsequent sequence analysis was restricted to M13 clones which did not contain sequence complementary to this probe. In this manner, two cDNA clones from independent PCR amplifications were identified that encoded identical V_L regions. Nucleotide sequence analysis of random V_H region PCR products revealed a single species of V_H region cDNA. Two V_H cDNA clones from independent PCR amplifications were found to contain identical sequences except that the codon of residue 14 encoded proline [CCT] in one clone while the second clone encoded leucine [OTT] at the same position.

According to Kabat et al 1987, 524 of 595 sequenced V_H regions contain a proline residue at this position, while only 6 contain leucine. We have therefore chosen the proline-encoding clone for illustration (see below). As residue 14 lies well within the first V_H framework region and not in a CDR, it is unlikely to contribute directly to antigen binding, and the ambiguity at this position did not affect the subsequent reshaping strategy. Thus, we have not investigated this sequence ambiguity further.

The cDNA sequences and their predicted amino acid sequences are shown in Figures 1 and 4. As no additional V_L or V_H region-encoding clones were found, it was assumed that these sequences were derived from the CD4 antibody genes.

Construction of reshaped antibodies.

Our goal was to investigate the importance of selecting the appropriate human V region framework during reshaping. Two reshaping strategies were employed.

First reshaping strategy.

In the first strategy, we created a reshaped antibody that incorporated the CDRs from the rat-derived CD4 antibody and the same human V region framework sequences that we had previously successfully used for the reshaped CAMPATH-1 antibody, namely an REI-based framework for the V_L region and an NEW-based framework for the V_H region (Reichmann et al, 1988). This was accomplished by oligonucleotide-directed in vitro mutagenesis of the six CDRs of the reshaped CAMPATH-1 antibody light and heavy chain cDNAs shown in Figures 2 and 5, respectively. The resultant reshaped CD4 antibody light chain (Figure 3) is called $CD4V_LREI$. Two versions of the NEW-based reshaped CD4 antibody heavy chain were created: $CD4V_HNEW\text{-}Thr^{30}$ (Figure 6) encoding a threonine residue at position 30 (in framework 1) and $CD4V_HNEW\text{-}Ser^{30}$ (Figure 7) encoding a serine residue at position 30. These two different versions were created because the successfully reshaped CAMPATH-1 antibody heavy chain bound antigen well whether position 30 encoded a threonine or serine residue (Reichmann et al, 1988), and we chose to test both possibilities in this case as well.

Second reshaping strategy

In the second reshaping strategy, we have reshaped the CD4 antibody V_H region to contain the V_H region framework sequences of the human antibody KOL. Of all known human antibody V_H regions, the overall amino acid sequence of the V_H region of KOL is most homologous to the rat CD4 antibody V_H region. The V_H regions of the human antibodies KOL and NEW are 66% and 42% homologous to the rat CD4 antibody V_H region, respectively.

Two versions of the KOL-based reshaped CD4 antibody heavy chain V region were created that differ by a single amino acid residue within the fourth framework region: $CD4V_HKOL\text{-}Pro^{113}$ (Figure 10) encodes a proline residue at position 113 and $CD4V_HKOL\text{-}Thr^{113}$ (Figure 12) encodes a threonine residue at position 113. $CD4V_HKOL\text{-}Pro^{113}$ is "true to form" in that its framework sequences are identical to those of the KOL antibody heavy chain V region (Figure 8).

Of all known human antibody V_L regions, the overall amino acid sequence of the V_L region of the human light chain NEW is most homologous (67%) to the rat CD4 antibody V_L region. Thus, the identical reshaped light chain, $CD4V_LREI$ (described above), that was expressed with the NEW-based reshaped CD4 antibody heavy chains $CD4V_HNEW\text{-}Thr^{30}$ and $CD4V_HNEW\text{-}Ser^{30}$, is also expressed with the KOL-based reshaped CD4 antibody heavy chains $CD4V_HKOL\text{-}Pro^{113}$ and $CD4V_HKOL\text{-}Thr^{113}$. This is advantageous because expression of the same reshaped light chain with different reshaped heavy chains allows for a direct functional comparison of each reshaped heavy chain.

To summarise, four different reshaped antibodies were created. The reshaped light chain of each antibody is called $CD4V_LREI$. The reshaped heavy chains of the antibodies are called $CD4V_HNEW\text{-}Thr^{30}$, $CD4V_HNEW\text{-}Ser^{30}$, $CD4V_HKOL\text{-}Pro^{113}$, and $CD4V_HKOL\text{-}Thr^{113}$, respectively. Each of the reshaped heavy chains contain the same human IgG1 constant region. As each reshaped antibody contains the same reshaped light chain, the name of a reshaped antibody's heavy chain shall be used below to refer to the whole antibody (heavy and light chain combination).

Relative affinities of the reshaped antibodies

The relative affinities of the reshaped antibodies were approximated by measuring their ability to bind to CD4 antigen-expressing cells at various antibody concentrations. FACS analysis determined the mean cellular fluorescence of the stained cells (Table 1).

It is clear from this analysis that the reshaped CD4 antibodies bind to CD4 antigen to varying degrees over a broad concentration range. Consider Experiment 1 of Table 1 first. Comparing $CD4V_HKOL\text{-}Thr^{113}$ antibody to $CD4V_HNEW\text{-}Thr^{30}$ antibody, it is clear that both antibodies bind $CD4^+$ cells when compared to the control, reshaped CAMPATH-1 antibody. However, $CD4V_HKOL\text{-}Thr^{113}$ antibody binds $CD4^+$ cells with far greater affinity than $CD4V_HNEW\text{-}Thr^{30}$ antibody. The lowest concentration of $CD4V_HKOL\text{-}Thr^{113}$ antibody tested (2.5 ug/ml) gave a mean cellular fluorescence nearly equivalent to that of the highest concentration of $CD4V_HNEW\text{-}Thr^{30}$ antibody tested (168 ug/ml). Experiment 2 demonstrates that $CD4V_HNEW\text{-}Ser^{30}$ antibody may bind $CD4^+$ cells somewhat better than $CD4V_HNEW\text{-}Thr^{30}$. Only 2.5 ug/ml $CD4V_HNEW\text{-}Ser^{30}$ antibody is required to give a mean cellular fluorescence nearly equivalent to 10 ug/ml $CD4V_HNEW\text{-}Thr^{30}$ antibody. Experiment 3 demonstrates that $CD4V_HKOL\text{-}Thr^{113}$ antibody may bind $CD4^+$ cells somewhat better than $CD4V_HKOL\text{-}Pro^{113}$ antibody.

From these assays, it is clear that the KOL-based reshaped antibodies are far superior to the NEW-based reshaped antibodies with regards to affinity towards $CD4^+$ cells. Also, there is a lesser difference, if any, between $CD4V_HNEW\text{-}Thr^{30}$ antibody and $CD4V_HNEW\text{-}Ser^{30}$ antibody, and likewise between $CD4V_HKOL\text{-}Thr^{113}$ antibody and $CD4V_HKOL\text{-}Pro^{113}$ antibody. A ranking of these reshaped antibodies can thus be derived based on their relative affinities for CD4+ cells:

$$CD4V_HKOL\text{-}Thr^{113} > CD4V_HKOL\text{-}Pro^{113} >> CD4V_HNEW\text{-}Ser^{30} > CD4V_HNEW\text{-}Thr^{30}$$

It should be restated that each of the reshaped CD4 antibodies used in the above experiments have the identical heavy chain constant regions, and are associated with identical reshaped light chains. Thus observed differences of binding to CD4+ cells must be due to differences in their heavy chain V regions.

Relative avidities of the rat YNB46.1.8 antibody and the reshaped $CD4V_HKOL\text{-}Thr^{113}$ antibody

The relative avidities of the rat YNB46.1.8 antibody and the reshaped $CD4V_HKOL\text{-}Thr^{113}$ antibody were estimated by ELISA. In this assay, the ability of each antibody to inhibit the binding of biotinylated $CD4V_HKOL\text{-}Thr^{113}$ antibody to soluble recombinant CD4 antigen was determined. Results of an experiment are shown in Figure 13. The inhibition of binding of biotinylated $CD4V_HKOL\text{-}Thr^{113}$ antibody was linear for both the unlabeled $CD4V_HKOL\text{-}Thr^{113}$ and YNB46.1.8 antibodies near the optical density of 0.3. The concentrations of $CD4V_HKOL\text{-}Thr^{113}$ and YNB46.1.8 antibodies that give an optical density of 0.3 are 28.7 and 1.56 ug/ml, respectively. Thus the avidity of the YNB46.1.8

antibody can be estimated to be 28.7/1.56 or about 18 times better than that of $CD4V_HKOL\text{-}Thr^{113}$ antibody. It should be noted that this experiment only provides a rough approximation of relative avidities, not affinities. The rat YNB46.1.8 antibody contains a different constant region than that of the $CD4V_HKOL\text{-}Thr^{113}$ antibody, and this could affect how well the antibodies bind CD4 antigen, irrespective of their actual affinities for CD4 antigen. The actual affinity of the reshaped antibodies for CD4 antigen may be greater, lesser, or the same as the YNB46.1.8 antibody. The other reshaped antibodies $CD4V_HKOL\text{-}Pro^{113}$, $CD4V_HNEW\text{-}Ser^{30}$, and $CD4V_HNEW\text{-}Thr^{30}$ have not yet been tested in this assay.

Table 1.

Mean cellular fluorescence of $CD4^+$ cells stained with reshaped antibodies		
Reshaped Antibody	Concentration (μg/ml)	Mean cellular Fluorescence
Experiment 1.		
$CD4V_HKOL\text{-}Thr^{113}$	113	578.0
$CD4V_HKOL\text{-}Thr^{113}$	40	549.0
$CD4V_HKOL\text{-}Thr^{113}$	10	301.9
$CD4V_HKOL\text{-}Thr^{113}$	2.5	100.5
$CD4V_HNEW\text{-}Thr^{30}$	168	97.0
$CD4V_HNEW\text{-}Thr^{30}$	40	40.4
$CD4V_HNEW\text{-}Thr^{30}$	10	18.7
$CD4V_HNEW\text{-}Thr^{30}$	2.5	10.9
CAMPATH-1	100	11.6
CAMPATH-1	40	9.4
CAMPATH-1	10	9.0
CAMPATH-1	2.5	8.6
CONTROL	----	9.0
Experiment 2.		
$CD4V_HNEW\text{-}Thr^{30}$	168	151.3
$CD4V_HNEW\text{-}Thr^{30}$	40	81.5
$CD4V_HNEW\text{-}Thr^{30}$	10	51.0
$CD4V_HNEW\text{-}Thr^{30}$	2.5	39.3
$CD4V_HNEW\text{-}Ser^{30}$	160	260.2
$CD4V_HNEW\text{-}Ser^{30}$	40	123.5
$CD4V_HNEW\text{-}Ser^{30}$	10	68.6
$CD4V_HNEW\text{-}Ser^{30}$	2.5	49.2
CONTROL	----	35.8
Experiment 3.		
$CD4V_HKOL\text{-}Pro^{113}$	100	594.9
$CD4V_HKOL\text{-}Pro^{113}$	40	372.0
$CD4V_HKOL\text{-}Pro^{113}$	10	137.7
$CD4V_HKOL\text{-}Pro^{113}$	2.5	48.9
$CD4V_HKOL\text{-}Thr^{113}$	100	696.7
$CD4V_HKOL\text{-}Thr^{113}$	40	631.5
$CD4V_HKOL\text{-}Thr^{113}$	10	304.1
$CD4V_HKOL\text{-}Thr^{113}$	2.5	104.0
CONTROL	----	12.3

Claims

Claims for the following Contracting States : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

1. A process for the preparation of an antibody chain in which complementarity determining regions (CDRs) of the variable domain of the antibody chain are derived from a first mammalian species and the framework of the variable domain and, if present, the or each constant domain of the antibody chain are derived from a second different mammalian species, which process comprises:

   (i) mutating the framework-encoding regions of DNA encoding a variable domain of an antibody chain of the said first species such that the mutated framework-encoding regions encode the said framework derived from the said second species; and
   (ii) expressing the said antibody chain utilising the mutated DNA from step (i);

   the mutation in step (i) being such that an antibody incorporating the antibody chain expressed in step (ii) retains the binding capability of the antibody from which the CDRs are derived.

2. A process according to claim 1, wherein the framework-encoding regions of DNA encoding the variable domain of an antibody heavy chain are mutated in step (i).

3. A process according to Claim 1 or 2, wherein the framework-encoding regions of DNA encoding the variable domain of an antibody light chain are mutated in step (i).

4. A process according to any one of the preceding claims, wherein the said first species is rat or mouse.

5. A process according to any one of the preceding claims, wherein the said second species is human.

6. A process according to any one of the preceding claims, comprising:

   (a) determining the nucleotide and predicted amino acid sequence of a variable domain of a selected antibody chain of the said first species;
   (b) determining the antibody framework to which the framework of the said domain is to be altered;
   (c) mutating the framework-encoding regions of DNA encoding the said variable domain such that the mutated framework-encoding regions encode the framework determined upon in step (b).
   (d) linking the mutated DNA obtained in step (c) to DNA encoding a constant domain of the said second species and cloning the DNA into an expression vector; and
   (e) introducing the expression vector into a compatible host cell and culturing the host cell under such conditions that antibody chain is expressed.

7. A process according to claim 6, in which about the most homologous framework of an antibody chain of a different species is selected in step (b) as the framework to which the framework of the said variable domain is to be altered.

8. A process according to any one of the preceding claims, wherein the antibody of the said first species is a CD4 antibody.

9. A process according to any one of the preceding claims, wherein the said antibody chain is co-expressed with a complementary antibody chain and antibody comprising the said two chains is recovered.

10. An antibody which is capable of binding to human CD4 antigen, in which the CDRs of the light chain of the antibody have the amino acid sequences:

    CDR1: LASEDIYSDLA
    CDR2: NTDTLQN
    CDR3: QQYNNYPWT

    in which the CDRs of the heavy chain of the antibody have the amino acid sequences:

CDR1: NYGMA
CDR2: TISHDGSDTYFRDSVKG
CDR3: QGTIAGIRH, and

in which the framework of the variable domain and, if present, the or each constant domain of each chain are derived from a mammalian non-rat species.

11. An antibody according to claim 10, in which the mammalian non-rat species is human.

12. An antibody according to claim 11, in which the variable domain framework of the heavy chain is homologous to the heavy chain variable domain framework of the protein KOL.

13. An antibody according to claim 12, in which the heavy chain variable region has the amino acid sequence shown in the upper line in Figure 10 or 12.

14. An antibody according to claim 11, in which the variable domain framework of the heavy chain is homologous to the heavy chain variable domain framework of the protein NEW.

15. An antibody according to claim 14, in which the heavy chain variable region has the amino acid sequence shown in the upper line of Figure 6 or 7.

16. An antibody according to any one of claims 11 to 15, in which the variable domain framework of the light chain is homologous to the variable domain framework of the protein REI.

17. An antibody according to claim 16, in which the light chain has the amino acid sequence shown in the upper line of Figure 3.

18. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an antibody as claimed in any one of claims 10 to 17.

Claims for the following Contracting States : ES, GR

1. A process for the preparation of an antibody chain in which complementarity determining regions (CDRs) of the variable domain of the antibody chain are derived from a first mammalian species and the framework of the variable domain and, if present, the or each constant domain of the antibody chain are derived from a second different mammalian species, which process comprises:

(i) mutating the framework-encoding regions of DNA encoding a variable domain of an antibody chain of the said first species such that the mutated framework-encoding regions encode the said framework derived from the said second species; and
(ii) expressing the said antibody chain utilising the mutated DNA from step (i);

the mutation in step (i) being such that an antibody incorporating the antibody chain expressed in step (ii) retains the binding capability of the antibody from which the CDRs are derived.

2. A process according to Claim 1, wherein the framework-encoding regions of DNA encoding the variable domain of an antibody heavy chain are mutated in step (i).

3. A process according to Claim 1 or 2, wherein the framework-encoding regions of DNA encoding the variable domain of an antibody light chain are mutated in step (i).

4. A process according to any one of the preceding claims, wherein the said first species is rat or mouse.

5. A process according to any one of the preceding claims, wherein the said second species is human.

6. A process according to any one of the preceding claims, comprising:

(a) determining the nucleotide and predicted amino acid sequence of a variable domain of a selected antibody chain of the said first species;
(b) determining the antibody framework to which the framework of the said domain is to be altered;
(c) mutating the framework-encoding regions of DNA encoding the said variable domain such that the mutated framework-encoding regions encode the framework determined upon in step (b);
(d) linking the mutated DNA obtained in step (c) to DNA encoding a constant domain of the said second species and cloning the DNA into an expression vector; and
(e) introducing the expression vector into a compatible host cell and culturing the host cell under such conditions that antibody chain is expressed.

7. A process according to claim 6, in which about the most homologous framework of an antibody chain of a different species is selected in step (b) as the framework to which the framework of the said variable domain is to be altered.

8. A process according to any one of the preceding claims, wherein the antibody of the said first species is a CD4 antibody.

9. A process according to any one of the preceding claims, wherein the said antibody chain is co-expressed with a complementary antibody chain and antibody comprising the said two chains is recovered.

10. A process for the production of an antibody which is capable of binding to human CD4 antigen, in which the CDRs of the light chain of the antibody have the amino acid sequences:

CDR1: LASEDIYSDLA
CDR2: NTDTLQN
CDR3: QQYNNYPWT

in which the CDRs of the heavy chain of the antibody have the amino acid sequences:

CDR1: NYGMA
CDR2: TISHDGSDTYFRDSVKG
CDR3: QGTIAGIRH, and

in which the framework of the variable domain and, if present, the or each constant domain of each chain are derived from a mammalian non-rat species, which comprises expressing DNA encoding the antibody in a suitable cell line.

11. A process according to claim 10, in which the mammalian non-rat species is human.

12. A process according to claim 11, in which the variable domain framework of the heavy chain is homologous to the heavy chain variable domain framework of the protein KOL.

13. A process according to claim 12, in which the heavy chain variable region has the amino acid sequence shown in the upper line in Figure 10 or 12.

14. A process according to claim 11, in which the variable domain framework of the heavy chain is homologous to the heavy chain variable domain framework of the protein NEW.

15. A process according to claim 14, in which the heavy chain variable region has the amino acid sequence shown in the upper line of Figure 6 or 7.

16. A process according to any one of claims 11 to 15, in which the variable domain framework of the light chain is homologous to the variable domain framework of the protein REI.

17. A process according to claim 16, in which the light chain has the amino acid sequence shown in the upper line of Figure 3.

18. A process for the production of a pharmaceutical composition which comprises formulating a pharmaceutically acceptable carrier or diluent with, as active ingredient, an antibody produced by a process as claimed in any one

of Claims 10 to 17.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

1. Verfahren zur Herstellung einer Antikörperkette, in der die Komplementarität-bestimmenden Regionen (CDRs) der variablen Domäne der Antikörperkette von einer ersten Säugerart abgeleitet sind und die Gerüstregion der variablen Domäne und, sofern vorhanden, die oder jede konstante Domäne der Antikörperkette von einer zweiten anderen Säugerart stammen, wobei man

   (i) die die Gerüstregion codierenden Regionen der DNA, die eine variable Domäne einer Antikörperkette der ersten Art codiert, so mutiert, daß die mutierten, die Gerüstregion codierenden Regionen die von der zweiten Art abgeleitete Gerüstregion codieren; und
   (ii) die Antikörperkette exprimiert, wobei die mutierte DNA aus Stufe (i) verwendet wird;

   wobei die Mutation in Stufe (i) so ist, daß ein Antikörper, der die in Stufe (ii) exprimierte Antikörperkette umfaßt, die Bindungsfähigkeit des Antikörpers, von dem die CDRs abgeleitet sind, beibehält.

2. Verfahren nach Anspruch 1, wobei die die Gerüstregion codierenden Regionen der DNA, die die variable Domäne einer schweren Kette eines Antikörpers codiert, in Stufe (i) mutiert werden.

3. Verfahren nach Anspruch 1 oder 2, wobei die die Gerüstregion codierenden Regionen der DNA, die die variable Domäne einer leichten Kette eines Antikörpers codiert, in Stufe (i) mutiert werden.

4. Verfahren nach einem der vorstehenden Ansprüche, wobei die erste Art eine Ratte oder eine Maus ist.

5. Verfahren nach einem der vorstehenden Ansprüche, wobei die zweite Art der Mensch ist.

6. Verfahren nach einem der vorstehenden Ansprüche, wobei man

   (a) die Nucleotid- und vorhergesagte Aminosäuresequenz einer variablen Domäne einer ausgewählten Antikörperkette der ersten Art bestimmt;
   (b) die Antikörpergerüstregion bestimmt, gegenüber der die Gerüstregion der Domäne verändert werden soll;
   (c) die die Gerüstregion codierenden Regionen der DNA, die die variable Domäne codiert, so mutiert, daß die mutierten, die Gerüstregion codierenden Regionen die in Stufe (b) bestimmte Gerüstregion codieren;
   (d) die in Stufe (c) erhaltene mutierte DNA an die DNA knüpft, die eine konstante Domäne der zweiten Art codiert, und die DNA in einem Expressionsvektor cloniert; und
   (e) den Expressionsvektor in eine kompatible Wirtszelle einschleust und die Wirtszelle unter solchen Bedingungen züchtet, daß die Antikörperkette exprimiert wird.

7. Verfahren nach Anspruch 6, wobei die Gerüstregion mit etwa der meisten Homologie einer Antikörperkette einer anderen Art in Stufe (b) als die Gerüstregion ausgewählt wird, gegenüber der die variable Domäne verändert werden soll.

8. Verfahren nach einem der vorstehenden Ansprüche, wobei der Antikörper der ersten Art ein CD4-Antikörper ist.

9. Verfahren nach einem der vorstehenden Ansprüche, wobei die Antikörperkette mit einer komplementären Antikörperkette gleichzeitig exprimiert wird und ein Antikörper, der die zwei Ketten umfaßt, isoliert wird.

10. Antikörper, der an menschliches CD4-Antigen binden kann, wobei die CDRs der leichten Kette des Antikörpers die Aminosäuresequenzen besitzen:

    CDR1: LASEDIYSDLA
    CDR2: NTDTLQN
    CDR3: QQYNNYPWT

und wobei die CDRs der schweren Kette des Antikörpers die Aminosäuresequenzen besitzen:

CDR1: NYGMA
CDR2: TISHDGSDTYFRDSVKG
CDR3: QGTIAGIRH

und wobei die Gerüstregion der variablen Domäne und, sofern vorhanden, die oder jede konstante Domäne jeder Kette von einer Nicht-Ratten-Säugerart abgeleitet sind.

11. Antikörper nach Anspruch 10, wobei die Nicht-Ratten-Säugerart der Mensch ist.

12. Antikörper nach Anspruch 11, wobei die Gerüstregion der variablen Domäne der schweren Kette der Gerüstregion der variablen Domäne der schweren Kette des Proteins KOL homolog ist.

13. Antikörper nach Anspruch 12, wobei die variable Region der schweren Kette die Aminosäuresequenz, die in der obersten Zeile der Fig. 10 oder 12 gezeigt ist, besitzt.

14. Antikörper nach Anspruch 11, wobei die Gerüstregion der variablen Domäne der schweren Kette der Gerüstregion der variablen Domäne der schweren Kette des Proteins NEW homolog ist.

15. Antikörper nach Anspruch 14, wobei die variable Region der schweren Kette die Aminosäuresequenz, die in der obersten Zeile von Fig. 6 oder 7 gezeigt ist, besitzt.

16. Antikörper nach einem der Ansprüche 11 bis 15, wobei die Gerüstregion der variablen Domäne der leichten Kette der Gerüstregion der variablen Domäne des Proteins REI homolog ist.

17. Antikörper nach Anspruch 16, wobei die leichte Kette die Aminosäuresequenz, die in der obersten Zeile der Fig. 3 gezeigt ist, besitzt.

18. Pharmazeutisches Präparat, umfassend einen pharmazeutisch verträglichen Träger oder ein pharmazeutisch verträgliches Verdünnungsmittel und als Wirkstoff einen Antikörper nach einem der Ansprüche 10 bis 17.

Patentansprüche für folgende Vertragsstaaten : ES, GR

1. Verfahren zur Herstellung einer Antikörperkette, in der die Komplementarität-bestimmenden Regionen (CDRs) der variablen Domäne der Antikörperkette von einer ersten Säugerart abgeleitet sind und die Gerüstregion der variablen Domäne und, sofern vorhanden, die oder jede konstante Domäne der Antikörperkette von einer zweiten anderen Säugerart stammen, wobei man

(i) die die Gerüstregion codierenden Regionen der DNA, die eine variable Domäne einer Antikörperkette der ersten Art codiert, so mutiert, daß die mutierten, die Gerüstregion codierenden Regionen die von der zweiten Art abgeleitete Gerüstregion codieren; und (ii) die Antikörperkette exprimiert, wobei die mutierte DNA aus Stufe (i) verwendet wird;

wobei die Mutation in Stufe (i) so ist, daß ein Antikörper, der die in Stufe (ii) exprimierte Antikörperkette umfaßt, die Bindungsfähigkeit des Antikörpers, von dem die CDRs abgeleitet sind, beibehält.

2. Verfahren nach Anspruch 1, wobei die die Gerüstregion codierenden Regionen der DNA, die die variable Domäne einer schweren Kette eines Antikörpers codiert, in Stufe (i) mutiert werden.

3. Verfahren nach Anspruch 1 oder 2, wobei die die Gerüstregion codierenden Regionen der DNA, die die variable Domäne einer leichten Kette eines Antikörpers codiert, in Stufe (i) mutiert werden.

4. Verfahren nach einem der vorstehenden Ansprüche, wobei die erste Art eine Ratte oder eine Maus ist.

5. Verfahren nach einem der vorstehenden Ansprüche, wobei die zweite Art der Mensch ist.

6. Verfahren nach einem der vorstehenden Ansprüche, wobei man

(a) die Nucleotid- und vorhergesagte Aminosäuresequenz einer variablen Domäne einer ausgewählten Antikörperkette der ersten Art bestimmt;
(b) die Antikörpergerüstregion bestimmt, gegenüber der die Gerüstregion der Domäne verändert werden soll;
(c) die die Gerüstregion codierenden Regionen der DNA, die die variable Domäne codiert, so mutiert, daß die mutierten, die Gerüstregion codierenden Regionen die in Stufe (b) bestimmte Gerüstregion codieren;
(d) die in Stufe (c) erhaltene mutierte DNA an die DNA knüpft, die eine konstante Domäne der zweiten Art codiert, und die DNA in einem Expressionsvektor cloniert; und
(e) den Expressionsvektor in eine kompatible Wirtszelle einschleust und die Wirtszelle unter solchen Bedingungen züchtet, daß die Antikörperkette exprimiert wird.

7. Verfahren nach Anspruch 6, wobei die Gerüstregion mit etwa der meisten Homologie einer Antikörperkette einer anderen Art in Stufe (b) als die Gerüstregion ausgewählt wird, gegenüber der die variable Domäne verändert werden soll.

8. Verfahren nach einem der vorstehenden Ansprüche, wobei der Antikörper der ersten Art ein CD4-Antikörper ist.

9. Verfahren nach einem der vorstehenden Ansprüche, wobei die Antikörperkette mit einer komplementären Antikörperkette gleichzeitig exprimiert wird und ein Antikörper, der die zwei Ketten umfaßt, isoliert wird.

10. Verfahren zur Produktion eines Antikörpers, der an menschliches CD4-Antigen binden kann, worin die CDRs der leichten Kette des Antikörpers die Aminosäuresequenzen

CDR1: LASEDIYSDLA
CDR2: NTDTLQN
CDR3: QQYNNYPWT

besitzen, worin die CDRs der schweren Kette des Antikörpers die Aminosäuresequenzen besitzen:

CDR1: NYGMA
CDR2: TISHDGSDTYFRDSVKG
CDR3: QGTIAGIRH

und worin die Gerüstregion der variablen Domäne und, sofern vorhanden, die oder jede konstante Domäne jeder Kette von einer Nicht-Ratten-Säugerart abgeleitet sind, wobei man die DNA, die den Antikörper codiert, in einer geeigneten Zellinie exprimiert.

11. Verfahren nach Anspruch 10, wobei die Nicht-Ratten-Säugerart der Mensch ist.

12. Verfahren nach Anspruch 11, wobei die Gerüstregion der variablen Domäne der schweren Kette der Gerüstregion der variablen Domäne der schweren Kette des Proteins KOL homolog ist.

13. Verfahren nach Anspruch 12, wobei die variable Region der schweren Kette die in der obersten Zeile der Fig. 10 oder 12 gezeigte Aminosäuresequenz besitzt.

14. Verfahren nach Anspruch 11, wobei die Gerüstregion der variablen Domäne der schweren Kette der Gerüstregion der variablen Domäne der schweren Kette des Proteins NEW homolog ist.

15. Verfahren nach Anspruch 14, wobei die variable Region der schweren Kette die Aminosäuresequenz, die in der obersten Zeile von Fig. 6 oder 7 gezeigt ist, besitzt.

16. Verfahren nach einem der Ansprüche 11 bis 15, wobei die Gerüstregion der variablen Domäne der leichten Kette der Gerüstregion der variablen Domäne des Proteins REI homolog ist.

17. Verfahren nach Anspruch 16, wobei die leichte Kette die in der obersten Zeile der Fig. 3 gezeigte Aminosäuresequenz besitzt.

18. Verfahren zur Herstellung eines pharmazeutischen Präparats, wobei man einen pharmazeutisch verträglichen Träger oder ein pharmazeutisch verträgliches Verdünnungsmittel mit einem nach einem der Ansprüche 10 bis 17 produzierten Antikörper als Wirkstoff formuliert.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, LI, DE, DK, FR, GB, IT, LU, NL, SE

1. Procédé de préparation d'une chaîne d'anticorps dans laquelle les régions de détermination de complémentarité (CDR) du domaine variable de la chaîne de l'anticorps dérivent d'une première espèce de mammifère et le cadre du domaine variable et, s'ils sont présents, le ou chaque domaine constant de la chaîne de l'anticorps dérivent d'une seconde espèce mammalienne différente, lequel procédé se caractérise en ce qu'il comprend :

   (i) la mutation de régions encodant le cadre d'ADN encodant un domaine variable d'une chaîne d'anticorps de ladite première espèce, en sorte que les régions encodant le cadre ayant subi la mutation encodent ledit cadre dérivé de ladite seconde espèce et
   (ii) l'expression de ladite chaîne d'anticorps utilisant l'ADN muté de l'étape (i),

   la mutation dans l'étape (i) étant telle qu'un anticorps incorporant la chaîne d'anticorps exprimée dans l'étape (ii) conserve le pouvoir de liaison de l'anticorps dont les RDC dérivent.

2. Procédé suivant la revendication 1, caractérisé en ce que les régions encodant le cadre d'ADN encodant le domaine variable d'une chaîne lourde d'anticorps sont mutées dans l'étape (i).

3. Procédé suivant la revendication 1 ou 2, caractérisé en ce que les régions encodant le cadre d'ADN encodant le domaine variable de chaîne légère d'anticorps sont mutées dans l'étape (i).

4. Procédé suivant l'une quelconque des revendications précédentes, caractérisé en ce que ladite première espèce est le rat ou la souris.

5. Procédé suivant l'une quelconque des revendications précédentes, caractérisé en ce que ladite seconde espèce est humaine.

6. Procédé suivant l'une quelconque des revendications précédentes, caractérisé en ce qu'on:

   (a) détermine le nucléotide et la séquence d'aminoacides prévue d'un domaine variable d'une chaîne d'anticorps choisie de ladite première espèce,
   (b) détermine le cadre d'anticorps auquel le cadre dudit domaine-variable doit être altéré,
   (c) mute les régions encodant le cadre d'ADN encodant ledit domaine variable en sorte que les régions encodant le cadre ayant subi la mutation encodent le cadre déterminé dans l'étape (b),
   (d) lie l'ADN muté obtenu dans l'étape (c) à l'ADN encodant un domaine constant de ladite seconde espèce et clonant l'ADN dans un vecteur d'expression et
   (e) introduit le vecteur d'expression dans une cellule hôte compatible et cultivant la cellule hôte dans des conditions telles que la chaîne d'anticorps soit exprimée.

7. Procédé suivant la revendication 6, caractérisé en ce qu'environ le cadre le plus homologue d'une chaîne d'anticorps d'une espèce différente est choisi dans l'étape (b) comme le cadre auquel le cadre du domaine variable précité doit être altéré.

8. Procédé suivant l'une quelconque des revendications précédentes, caractérisé en ce que l'anticorps de ladite première espèce est un anticorps CD4.

9. Procédé suivant l'une quelconque des revendications précédentes, caractérisé en ce que ladite chaîne d'anticorps est co-exprimée ou exprimée conjointement avec une chaîne d'anticorps complémentaire et l'anticorps comprenant lesdites deux chaînes est récupéré.

10. Anticorps qui est capable de se lier à un antigène CD4 humain, caractérisé en ce que les RDC de la chaîne légère de l'anticorps possèdent les séquences d'aminoacides qui suivent :

CDR1: LASEDIYSDLA
CDR2: NTDTLQN
CDR3: QQYNNYPWT,

dans lequel, les RDC de la chaîne lourde de l'anticorps possèdent les séquences d'aminoacides suivantes :

CDR1: NYGMA
CDR2: TISHDGSDTYFRDSVKG
CDR3: QGTIAGIRH, et

dans lequel le cadre du domaine variable et, pour autant que présents, le ou chaque domaine constant de chaque chaîne sont dérivés d'une espèce mammalienne non rat.

11. Anticorps suivant la revendication 10, caractérisé en ce que l'espèce mammalienne non rat est humaine.

12. Anticorps suivant la revendication 11, caractérisé en ce que le cadre du domaine variable de la chaîne lourde est homologue au cadre du domaine variable de la chaîne lourde de la protéine KOL.

13. Anticorps suivant la revendication 12, caractérisé en ce que la région variable de la chaîne lourde possède la séquence d'aminoacides montrée dans la ligne supérieure dans la figure 10 ou 12.

14. Anticorps suivant la revendication 11, caractérisé en ce que le cadre du domaine variable de la chaîne lourde est homologue au cadre du domaine variable de la chaîne lourde de la protéine NEW.

15. Anticorps suivant la revendication 14, caractérisé en ce que la région variable de la chaîne lourde possède la séquence d'aminoacides montrée dans la ligne supérieure de la figure 6 ou 7.

16. Anticorps suivant l'une quelconque des revendications 11 à 15, caractérisé en ce que le cadre du domaine variable de la chaîne légère est homologue au cadre du domaine variable de la protéine REI.

17. Anticorps suivant la revendication 16, caractérisé en ce que la chaîne légère possède la séquence d'aminoacides montrée dans la ligne supérieure de la figure 3.

18. Composition pharmaceutique, qui comprend un diluant, véhicule ou excipient pharmaceutiquement acceptable et, à titre d'ingrédient actif, un anticorps suivant l'une quelconque des revendications 10 à 17.

Revendications pour les Etats contractants suivants : ES, GR

1. Procédé de préparation d'une chaîne d'anticorps dans laquelle les régions de détermination de complémentarité (CDR) du domaine variable de la chaîne de l'anticorps dérivent d'une première espèce de mammifère et le cadre du domaine variable et, s'ils sont présents, le ou chaque domaine constant de la chaîne de l'anticorps dérivent d'une seconde espèce mammalienne différente, lequel procédé se caractérise en ce qu'il comprend :

(i) la mutation de régions encodant le cadre d'ADN encodant un domaine variable d'une chaîne d'anticorps de ladite première espèce, en sorte que les régions encodant le cadre ayant subi la mutation encodent ledit cadre dérivé de ladite seconde espèce et
(ii) l'expression de ladite chaîne d'anticorps utilisant l'ADN muté de l'étape (i),

la mutation dans l'étape (i) étant telle qu'un anticorps incorporant la chaîne d'anticorps exprimée dans l'étape (ii) conserve le pouvoir de liaison de l'anticorps dont les RDC dérivent.

2. Procédé suivant la revendication 1, caractérisé en ce que les régions encodant le cadre d'ADN encodant le domaine variable d'une chaîne lourde d'anticorps sont mutées dans l'étape (i).

3. Procédé suivant la revendication 1 ou 2, caractérisé en ce que les régions encodant le cadre d'ADN encodant le domaine variable de chaîne légère d'anticorps sont mutées dans l'étape (i).

4. Procédé suivant l'une quelconque des revendications précédentes, caractérisé en ce que ladite première espèce est le rat ou la souris.

5. Procédé suivant l'une quelconque des revendications précédentes, caractérisé en ce que ladite seconde espèce est humaine.

6. Procédé suivant l'une quelconque des revendications précédentes, caractérisé en ce qu'on:

(a) détermine le nucléotide et la séquence d'aminoacides prévue d'un domaine variable d'une chaîne d'anticorps choisie de ladite première espèce,
(b) détermine le cadre d'anticorps auquel le cadre dudit domaine variable doit être altéré,
(c) mute les régions encodant le cadre d'ADN encodant ledit domaine variable en sorte que les régions encodant le cadre ayant subi la mutation encodent le cadre déterminé dans l'étape (b),
(d) lie l'ADN muté obtenu dans l'étape (c) à l'ADN encodant un domaine constant de ladite seconde espèce et clonant l'ADN dans un vecteur d'expression et
(e) introduit le vecteur d'expression dans une cellule hôte compatible et cultivant la cellule hôte dans des conditions telles que la chaîne d'anticorps soit exprimée.

7. Procédé suivant la revendication 6, caractérisé en ce qu'environ le cadre le plus homologue d'une chaîne d'anticorps d'une espèce différente est choisi dans l'étape (b) comme le cadre auquel le cadre du domaine variable précité doit être altéré.

8. Procédé suivant l'une quelconque des revendications précédentes, caractérisé en ce que l'anticorps de ladite première espèce est un anticorps CD4.

9. Procédé suivant l'une quelconque des revendications précédentes, caractérisé en ce que ladite chaîne d'anticorps est co-exprimée ou exprimée conjointement avec une chaîne d'anticorps complémentaire et l'anticorps comprenant lesdites deux chaînes est récupéré.

10. Procédé de production d'un anticorps qui est capable de se lier à un antigène CD4 humain, caractérisé en ce que les RDC de la chaîne légère de l'anticorps possèdent les séquences d'aminoacides qui suivent :

CDR1: LASEDIYSDLA
CDR2: NTDTLQN
CDR3: QQYNNYPWT,

dans lequel, les RDC de la chaîne lourde de l'anticorps possèdent les séquences d'aminoacides suivantes :

CDR1: NYGMA
CDR2: TISHDGSDTYFRDSVKG
CDR3: QGTIAGIRH, et

dans lequel le cadre du domaine variable et, pour autant que présents, le ou chaque domaine constant de chaque chaîne sont dérivés d'une espèce mammalienne non rat, caractérisé en ce qu'il comprend l'expression de l'ADN encodant l'anticorps dans une lignée cellulaire convenable.

11. Procédé suivant la revendication 10, caractérisé en ce que l'espèce mammalienne non rat est humaine.

12. Procédé suivant la revendication 11, caractérisé en ce que le cadre du domaine variable de la chaîne lourde est homologue au cadre du domaine variable de la chaîne lourde de la protéine KOL.

13. Procédé suivant la revendication 12, caractérisé en ce que la région variable de la chaîne lourde possède la séquence d'aminoacides montrée dans la ligne supérieure dans la figure 10 ou 12.

14. Procédé suivant la revendication 11, caractérisé en ce que le cadre du domaine variable de la chaîne lourde est

homologue au cadre du domaine variable de la chaîne lourde de la protéine NEW.

15. Procédé suivant la revendication 14, caractérisé en ce que la région variable de la chaîne lourde possède la séquence d'aminoacides montrée dans la ligne supérieure de la figure 6 ou 7.

16. Procédé suivant l'une quelconque des revendications 11 à 15, caractérisé en ce que le cadre du domaine variable de la chaîne légère est homologue au cadre du domaine variable de la protéine REI.

17. Procédé suivant la revendication 16, caractérisé en ce que la chaîne légère possède la séquence d'aminoacides montrée dans la ligne supérieure de la figure 3.

18. Procédé de fabrication d'une composition pharmaceutique, qui comprend un diluant, véhicule ou excipient pharmaceutiquement acceptable et, à titre d'ingrédient actif, un anticorps suivant l'une quelconque des revendications 10 à 17.

FIG. 1

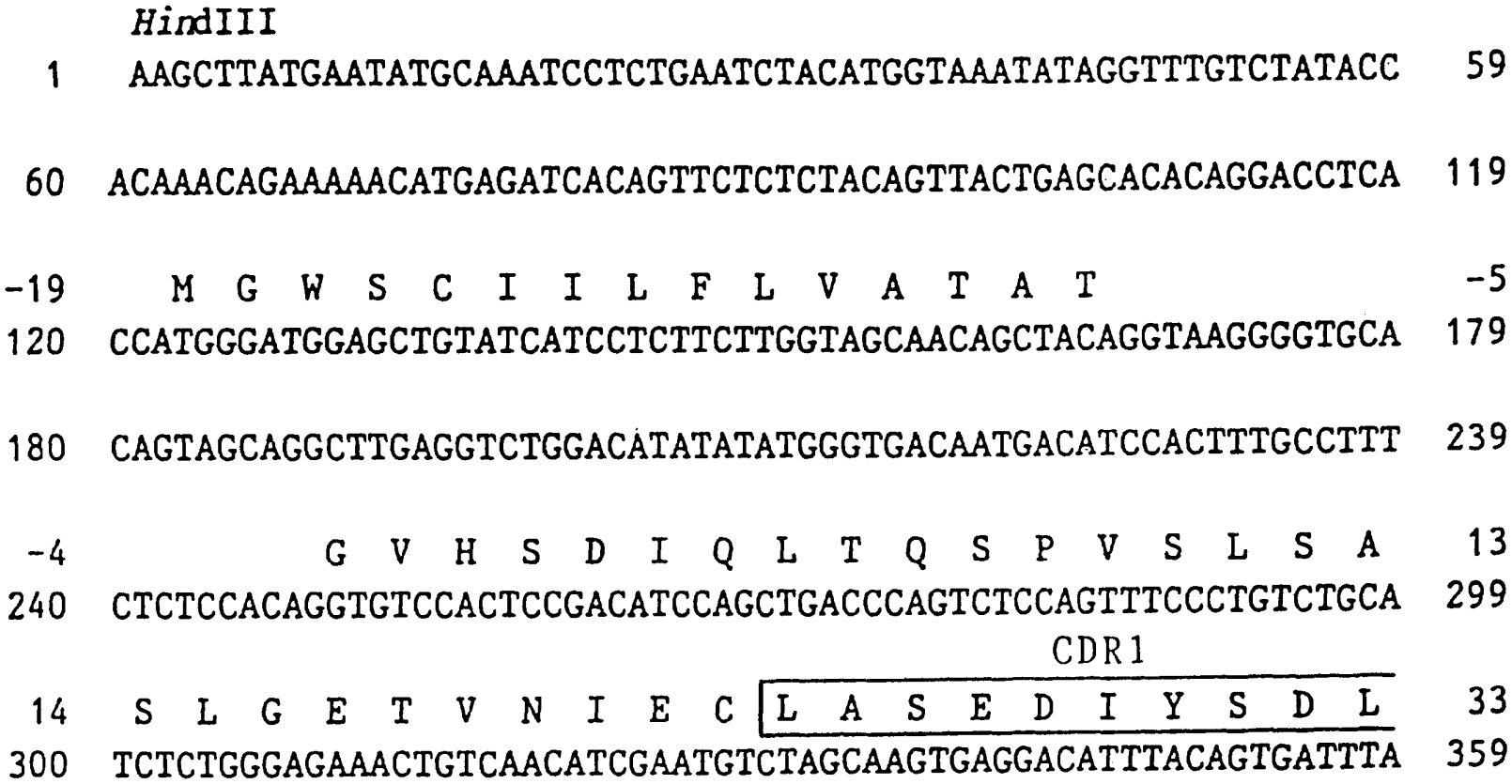

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FIG.1 (contd.)

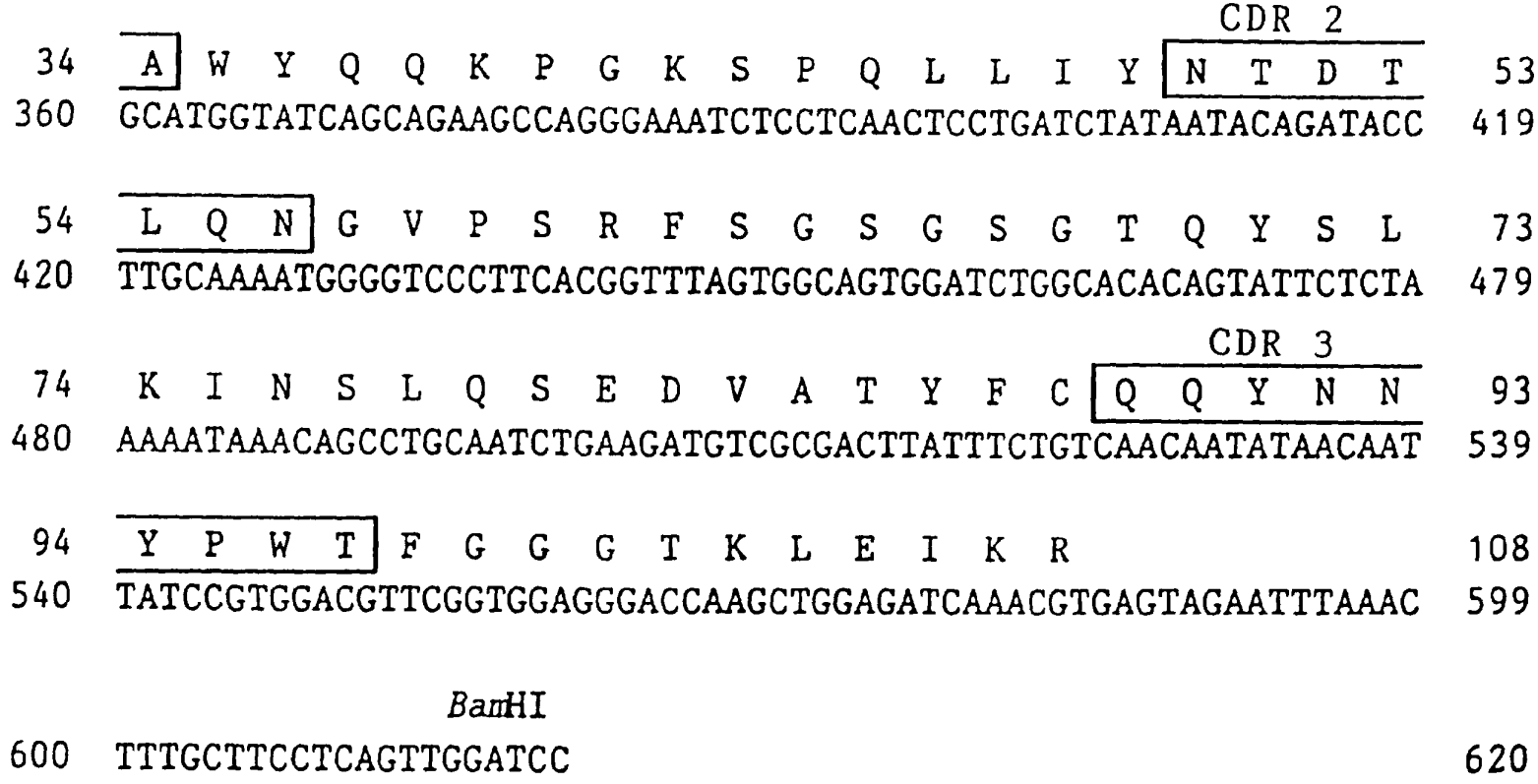
CDR 2
34 A W Y Q Q K P G K S P Q L L I Y N T D T 53
360 GCATGGTATCAGCAGAAGCCAGGGAAATCTCCTCAACTCCTGATCTATAATACAGATACC 419
54 L Q N G V P S R F S G S G S G T Q Y S L 73
420 TTGCAAAATGGGGTCCCTTCACGGTTTAGTGGCAGTGGATCTGGCACACAGTATTCTCTA 479
CDR 3
74 K I N S L Q S E D V A T Y F C Q Q Y N N 93
480 AAAATAAACAGCCTGCAATCTGAAGATGTCGCGACTTATTTCTGTCAACAATATAACAAT 539
94 Y P W T F G G G T K L E I K R 108
540 TATCCGTGGACGTTCGGTGGAGGGACCAAGCTGGAGATCAAACGTGAGTAGAATTTAAAC 599
BamHI
600 TTTGCTTCCTCAGTTGGATCC 620

FIG. 2

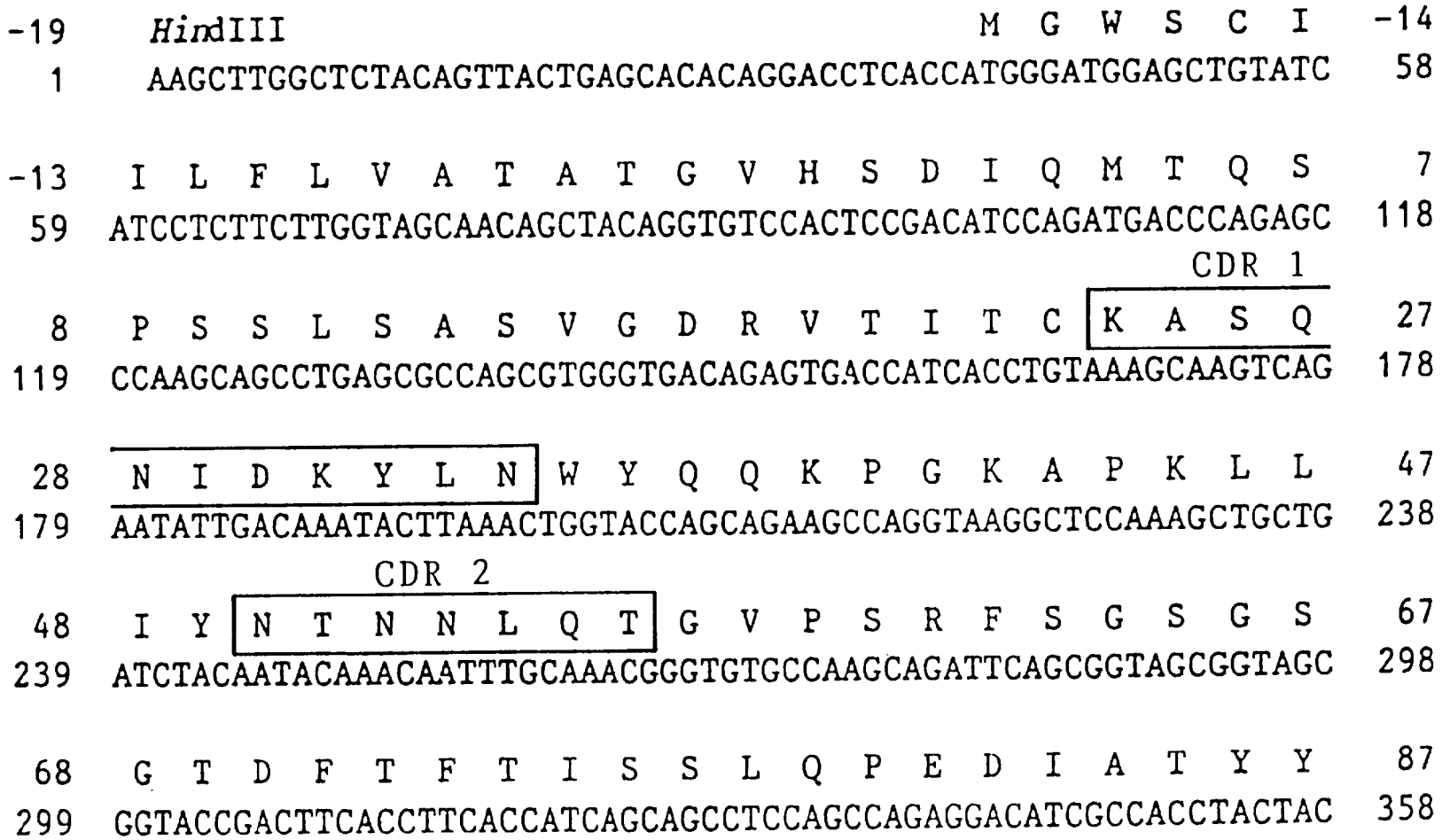

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FIG. 2(contd.)

CDR 3

```
 88   C  L  Q  H  I  S  R  P  R  T  F  G  Q  G  T  K  V  E  I  K   107
359   TGCTTGCAGCATATAAGTAGGCCGCGCACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA   418

108   R  T  V  A  A  P  S  V  F  I  F  P  P  S  D  E  Q  L  K  S   127
419   CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT   478

128   G  T  A  S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q   147
479   GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAG   538

148   W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D   167
539   TGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC   598

168   S  K  D  S  T  Y  S  L  S  S  T  L  T  L  S  K  A  D  Y  E   187
599   AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAG   658

188   K  H  K  V  Y  A  C  E  V  T  H  Q  G  L  S  S  P  V  T  K   207
659   AAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAG

208   S  F  N  R  G  E  C  Trm HindIII                              214
719   AGCTTCAACAGGGGAGAGTGTTAGAAGCTT                                 748
```

FIG. 3

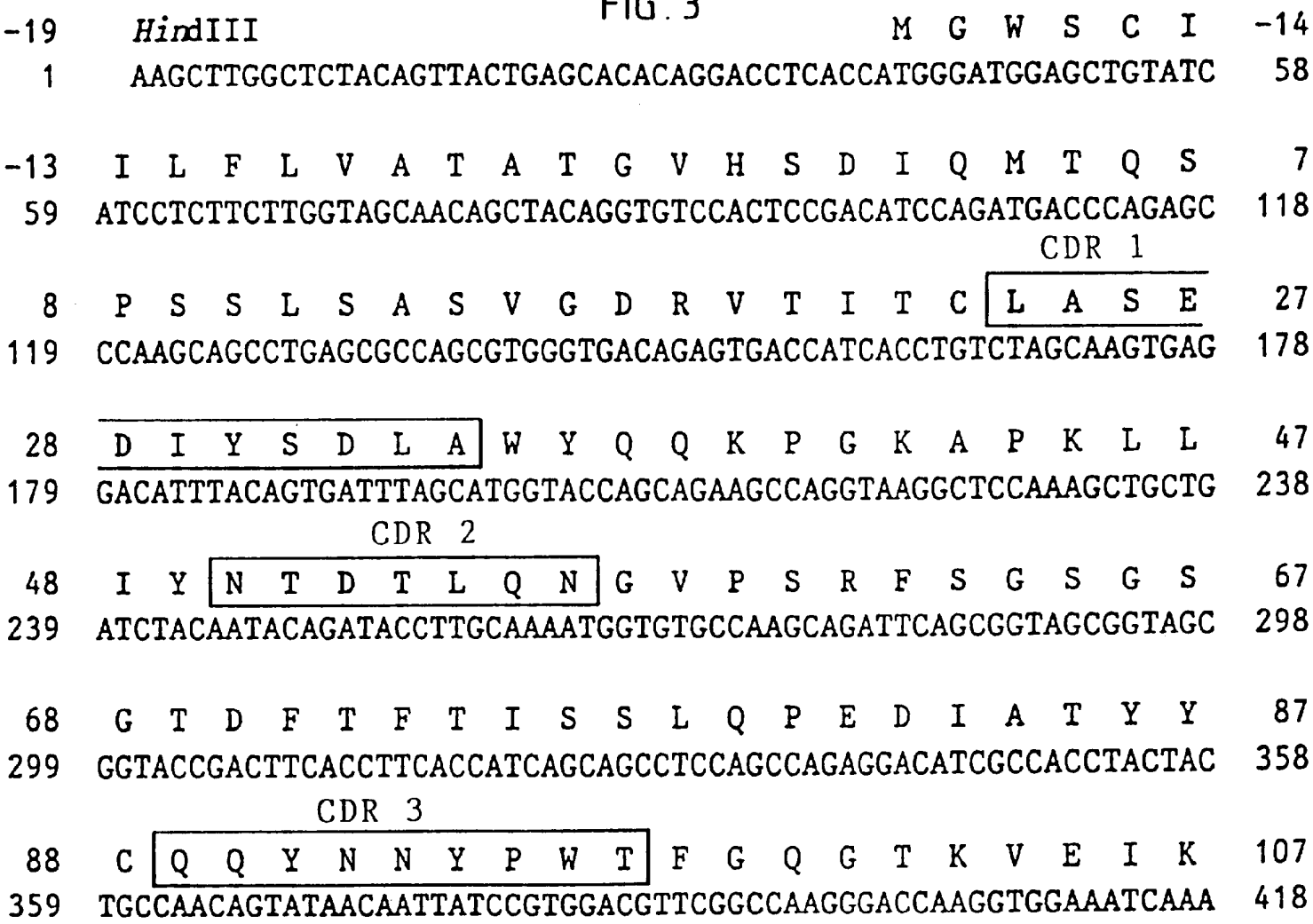

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FIG. 3 (contd.)

```
108   R  T  V  A  A  P  S  V  F  I  F  P  P  S  D  E  Q  L  K  S   127
419   CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCT   478

128   G  T  A  S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q   147
479   GGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAG   538

148   W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q  D   167
539   TGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC   598

168   S  K  D  S  T  Y  S  L  S  S  T  L  T  L  S  K  A  D  Y  E   187
599   AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAG   658

188   K  H  K  V  Y  A  C  E  V  T  H  Q  G  L  S  S  P  V  T  K   207
659   AAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAG   718

208   S  F  N  R  G  E  C  Trm  HindIII                            214
719   AGCTTCAACAGGGGAGAGTGTTAGAAGCTT                               748
```

FIG. 4

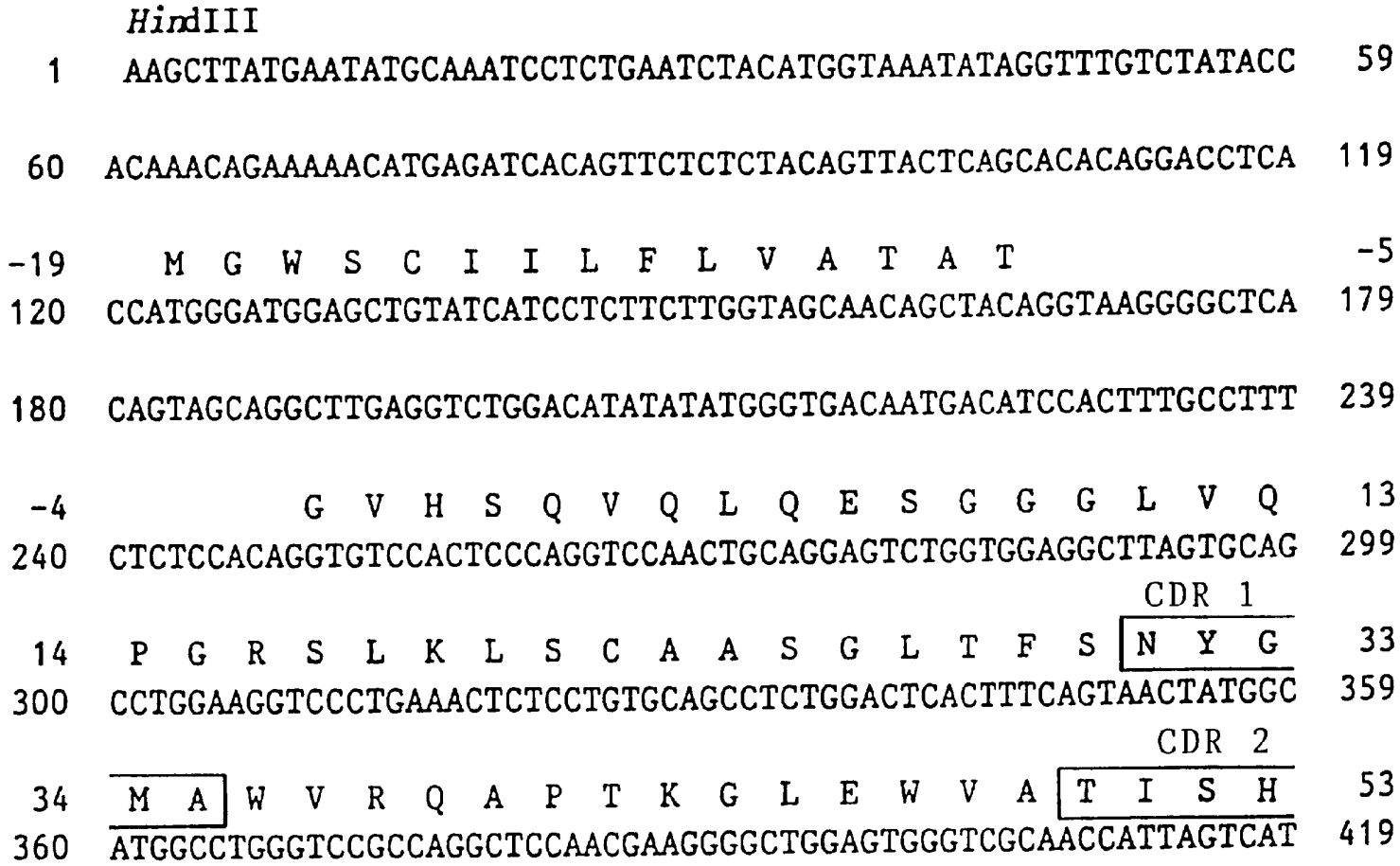

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FIG. 4 (contd.)

```
 54  D  G  S  D  T  Y  F  R  D  S  V  K  G  R  F  T  I  S  R  D   73
420  GATGGTAGTGACACTTACTTTCGAGACTCCGTGAAGGGCCGATTCACTATCTCCAGAGAT  479

 74  N  G  K  S  T  L  Y  L  Q  M  D  S  L  R  S  E  D  T  A  T   93
480  AATGGAAAAAGCACCCTATACCTGCAAATGGACAGTCTGAGGTCTGAGGACACGGCCACT  539

 94  Y  Y  C  A  R  Q  G  T  I  A  G  I  R  H  W  G  Q  G  T  T  113
540  TATTACTGTGCAAGACAAGGGACTATAGCAGGTATACGTCACTGGGGCCAAGGGACCACG  599

114  V  T  V  S  S                                               118
600  GTCACCGTCTCCTCAGGTGAGTCCTTACAACCTCTCTCTTCTATTCAGCTTAAATAGATT  659

660  TTACTGCATTTGTTGGGGGGGAAATGTGTGTATCTGAATTTCAGGTCATGAAGGACTAGG  719

720  GACACCTTGGGAGTCAGAAAGGGTCATTGGGAGCCCGGGCTGATGCAGACAGACATCCTC  779

                                    BamHI
780  AGCTCCCAGACTTCATGGCCAGAGATTTATAGGGATCC                        817
```


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FIG.5

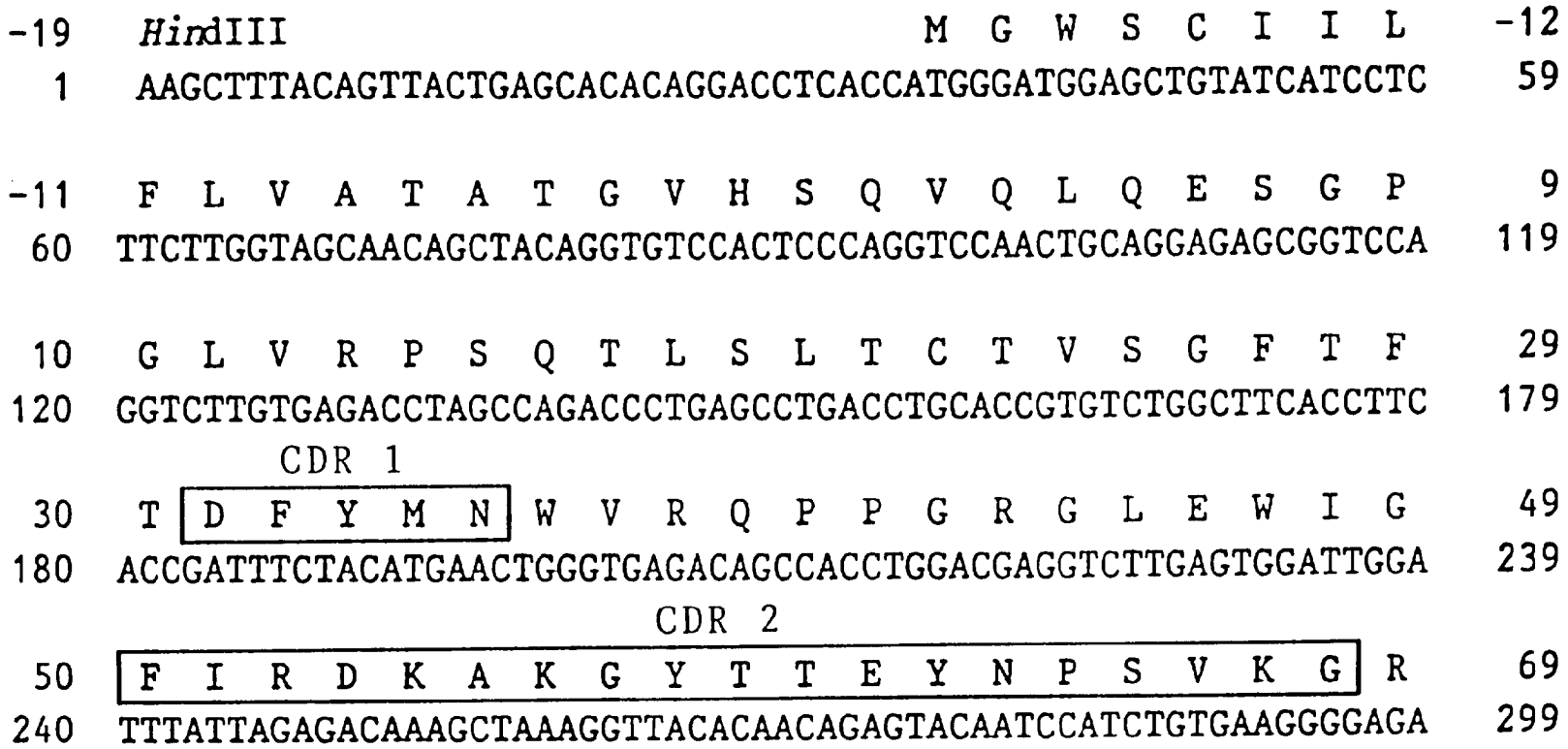

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FIG. 5 (contd.)

```
 70   V  T  M  L  V  D  T  S  K  N  Q  F  S  L  R  L  S  S  V  T    89
300   GTGACAATGCTGGTAGACACCAGCAAGAACCAGTTCAGCCTGAGACTCAGCAGCGTGACA   359
                                                  CDR 3
 90   A  A  D  T  A  V  Y  Y  C  A  R [E  G  H  T  A  A  P  F  D]   109
360   GCCGCCGACACCGCGGTCTATTATTGTGCAAGAGAGGGCCACACTGCTGCTCCTTTTGAT   419

110  [Y] W  G  Q  G  S  L  V  T  V  S  S  A  S  T  K  G  P  S  V   129
420   TACTGGGGTCAAGGCAGCCTCGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTC   479

130   F  P  L  A  P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L   149
480   TTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG   539

150   V  K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S   169
540   GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGC   599
```


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FIG.5 (contd.)

```
170   G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V    189
600   GGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG   659

190   V  T  V  P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K    209
660   GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAG   719

210   P  S  N  T  K  V  D  K  K  V  E  P  K  S  C  D  K  T  H  T    229
720   CCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACA   779

230   C  P  P  C  P  A  P  E  L  L  G  G  P  S  V  F  L  F  P  P    249
780   TGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCA   839

250   K  P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D    269
840   AAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGAC   899
```


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FIG. 5 (contd.)

```
270   V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H   289
900   GTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT   959

290   N  A  K  T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V   309
960   AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTC  1019

310   L  T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N   329
1020  CTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAAC  1079

330   K  A  L  P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E   349
1080  AAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA  1139

350   P  Q  V  Y  T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S  L   369
1140  CCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTG  1199
```


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FIG. 5 (contd.)

```
 370   T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G    389
1200   ACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG  1259

 390   Q  P  E  N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F    409
1260   CAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC  1319

 410   L  Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C    429
1320   CTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC  1379

 430   S  V  M  H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P    448
1380   TCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCG  1439

 449   G  K Trm                  HindIII                              450
1440   GGTAAATGAGTGCGACGGCCCCAAGCTT                                   1467
```


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FIG. 6

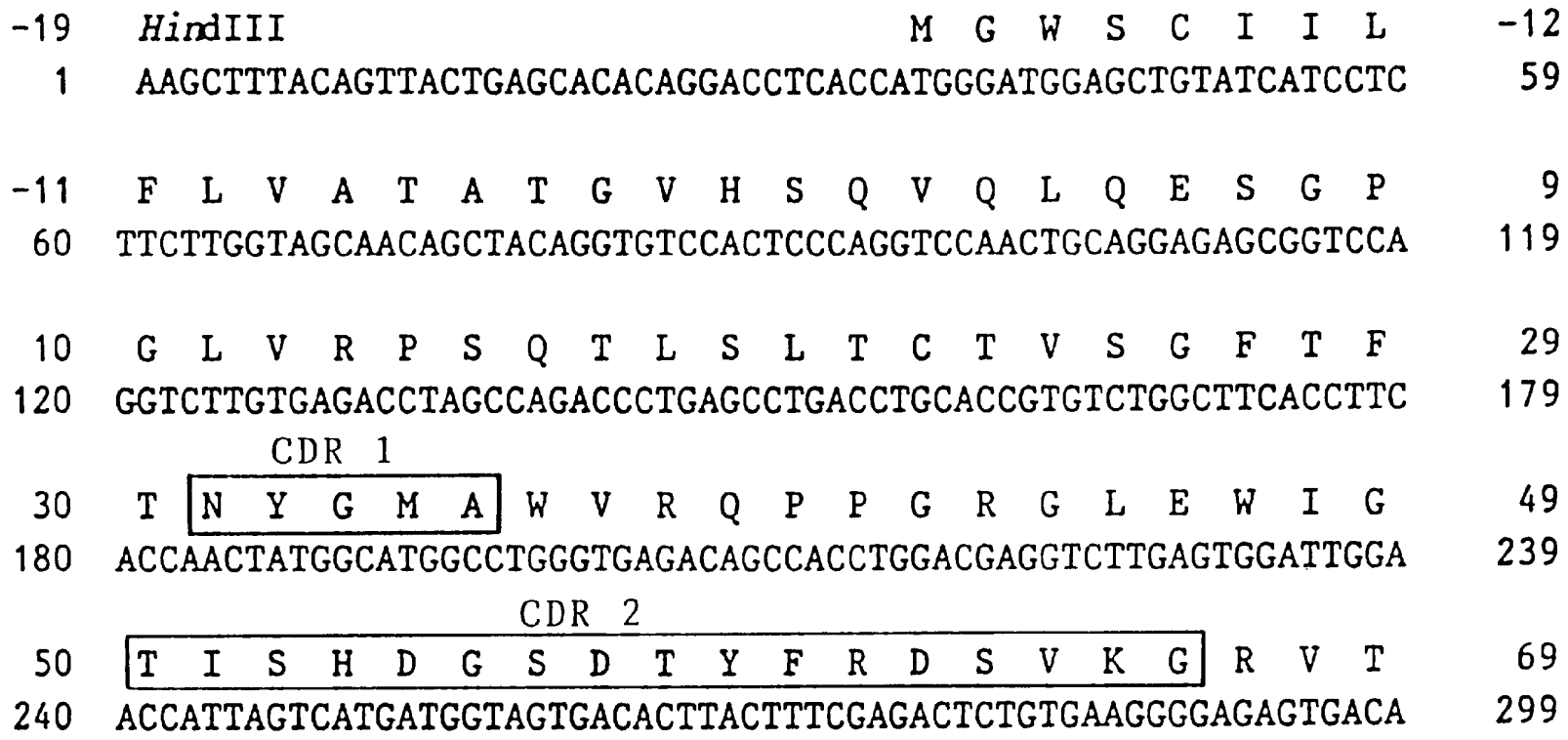

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FIG. 6 (contd.)

```
 70   M  L  V  D  T  S  K  N  Q  F  S  L  R  L  S  S  V  T  A  A     89
300   ATGCTGGTAGACACCAGCAAGAACCAGTTCAGCCTGAGACTCAGCAGCGTGACAGCCGCC  359
                                        CDR 3
 90   D  T  A  V  Y  Y  C  A  R  Q  G  T  I  A  G  I  R  H  W  G     109
360   GACACCGCGGTCTATTATTGTGCAAGACAAGGCACTATAGCTGGTATACGTCACTGGGGT  419

110   Q  G  S  L  V  T  V  S  S  A  S  T  K  G  P  S  V  F  P  L     129
420   CAAGGCAGCCTCGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTG  479

130   A  P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D     149
480   GCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC  539

150   Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S  G  V  H     169
540   TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC  599
```


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FIG. 6(contd.)

```
170   T  F  P  A  V  L  Q  S  S  G  L  Y  S  L  S  S  V  V  T  V    189
600   ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG    659

190   P  S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K  P  S  N    209
660   CCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC    719

210   T  K  V  D  K  K  V  E  P  K  S  C  D  K  T  H  T  C  P  P    229
720   ACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCG    779

230   C  P  A  P  E  L  L  G  G  P  S  V  F  L  F  P  P  K  P  K    249
780   TGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAG    839

250   D  T  L  M  I  S  R  T  P  E  V  T  C  V  V  V  D  V  S  H    269
840   GACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC    899
```


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FIG. 6 (contd.)

```
270   E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K    289
900   GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAG   959

290   T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V    309
960   ACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC   1019

310   L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L    329
1020  CTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC   1079

330   P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V    349
1080  CCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG   1139

350   Y  T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L    369
1140  TACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTG   1199
```


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FIG. 6 (contd.)

```
 370   V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E     389
1200   GTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG   1259

 390   N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S     409
1260   AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC   1319

 410   K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M     429
1320   AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG   1379

 430   H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P  G  K Trm   448
1380   CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA   1439

                    HindIII
1440   GTGCGACGGCCCCAAGCTT                                            1458
```


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FIG. 7

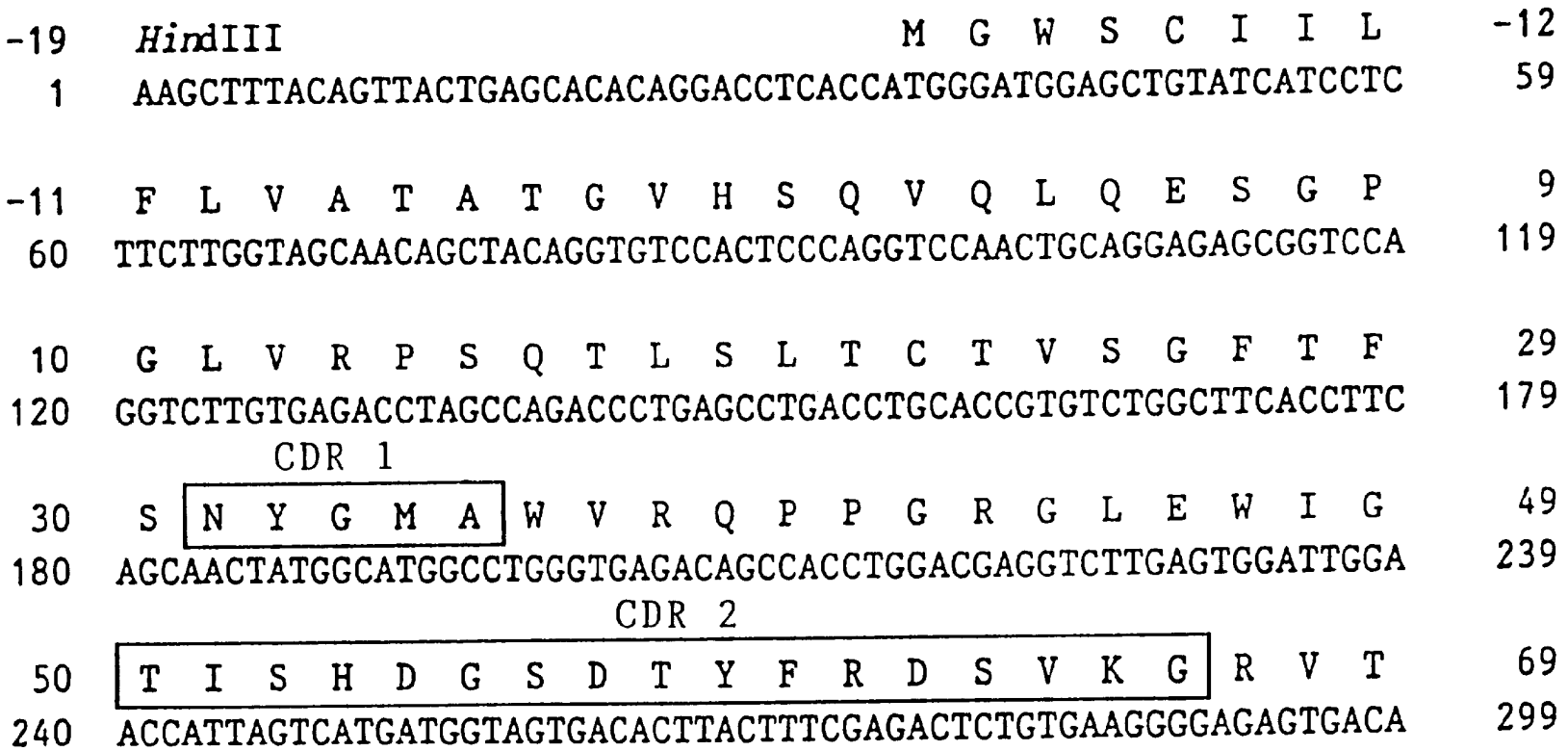
-19 HindIII M G W S C I I L -12
1 AAGCTTTACAGTTACTGAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTC 59
-11 F L V A T A T G V H S Q V Q L Q E S G P 9
60 TTCTTGGTAGCAACAGCTACAGGTGTCCACTCCCAGGTCCAACTGCAGGAGAGCGGTCCA 119
10 G L V R P S Q T L S L T C T V S G F T F 29
120 GGTCTTGTGAGACCTAGCCAGACCCTGAGCCTGACCTGCACCGTGTCTGGCTTCACCTTC 179
CDR 1
30 S N Y G M A W V R Q P P G R G L E W I G 49
180 AGCAACTATGGCATGGCCTGGGTGAGACAGCCACCTGGACGAGGTCTTGAGTGGATTGGA 239
CDR 2
50 T I S H D G S D T Y F R D S V K G R V T 69
240 ACCATTAGTCATGATGGTAGTGACACTTACTTTCGAGACTCTGTGAAGGGGAGAGTGACA 299

FIG. 7 (contd.)

```
 70   M  L  V  D  T  S  K  N  Q  F  S  L  R  L  S  S  V  T  A  A      89
300   ATGCTGGTAGACACCAGCAAGAACCAGTTCAGCCTGAGACTCAGCAGCGTGACAGCCGCC    359
                                        CDR 3
 90   D  T  A  V  Y  Y  C  A  R  Q  G  T  I  A  G  I  R  H  W  G      109
360   GACACCGCGGTCTATTATTGTGCAAGACAAGGCACTATAGCTGGTATACGTCACTGGGGT    419

110   Q  G  S  L  V  T  V  S  S  A  S  T  K  G  P  S  V  F  P  L      129
420   CAAGGCAGCCTCGTCACAGTCTCCTCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTG    479

130   A  P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L  V  K  D      149
480   GCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGAC    539

150   Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L  T  S  G  V  H      169
540   TACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCAC    599
```


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FIG. 7 (contd.)

170	T F P A V L Q S S G L Y S L S S V V T V	189
600	ACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTG	659
190	P S S S L G T Q T Y I C N V N H K P S N	209
660	CCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAAC	719
210	T K V D K K V E P K S C D K T H T C P P	229
720	ACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCG	779
230	C P A P E L L G G P S V F L F P P K P K	249
780	TGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAG	839
250	D T L M I S R T P E V T C V V V D V S H	269
840	GACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC	899

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FIG. 7 (contd.)

```
270   E  D  P  E  V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K    289
900   GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAG   959

290   T  K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L  T  V    309
960   ACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC   1019

310   L  H  Q  D  W  L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L    329
1020  CTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC   1079

330   P  A  P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P  Q  V    349
1080  CCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG   1139

350   Y  T  L  P  P  S  R  D  E  L  T  K  N  Q  V  S  L  T  C  L    369
1140  TACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTG   1199
```


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FIG. 7 (contd.)

```
370   V  K  G  F  Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E     389
1200  GTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG  1259

390   N  N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L  Y  S     409
1260  AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGC  1319

410   K  L  T  V  D  K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M     429
1320  AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG  1379

430   H  E  A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P  G  K Trm   448
1380  CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA  1439

                    HindIII
1440  GTGCGACGGCCCCAAGCTT                                            1458
```


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FIG.8

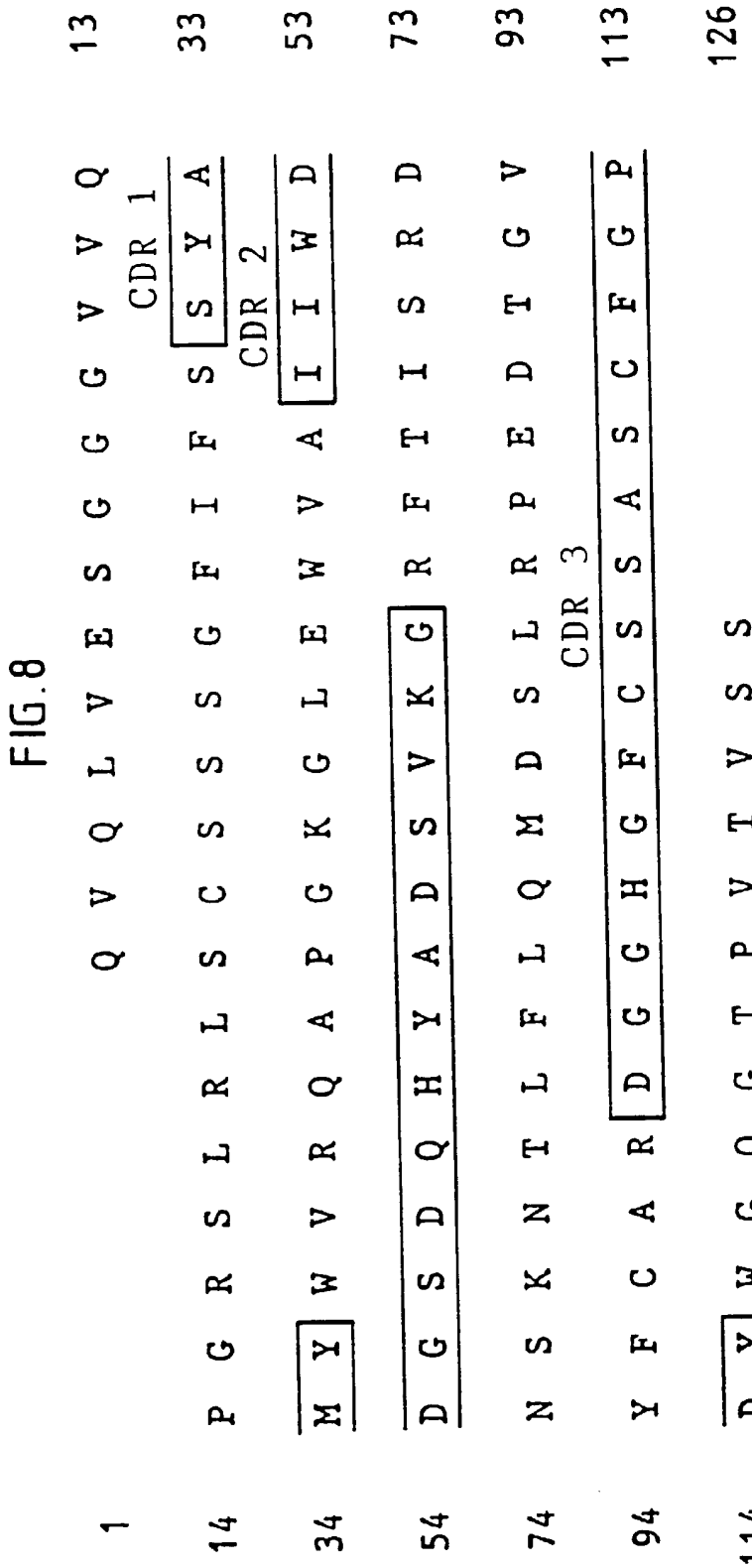
1
Q V Q L V E S G G G V V Q
13
CDR 1
14
P G R S L R L S C S S S G F I F S S Y A
33
CDR 2
34
M Y W V R Q A P G K G L E W V A I I W D
53
54
D G S D Q H Y A D S V K G R F T I S R D
73
CDR 3
74
N S K N T L F L Q M D S L R P E D T G V
93
94
Y F C A R D G G H G F C S S A S C F G P
113
114
D Y W G Q G T P V T V S S
126

FIG. 9

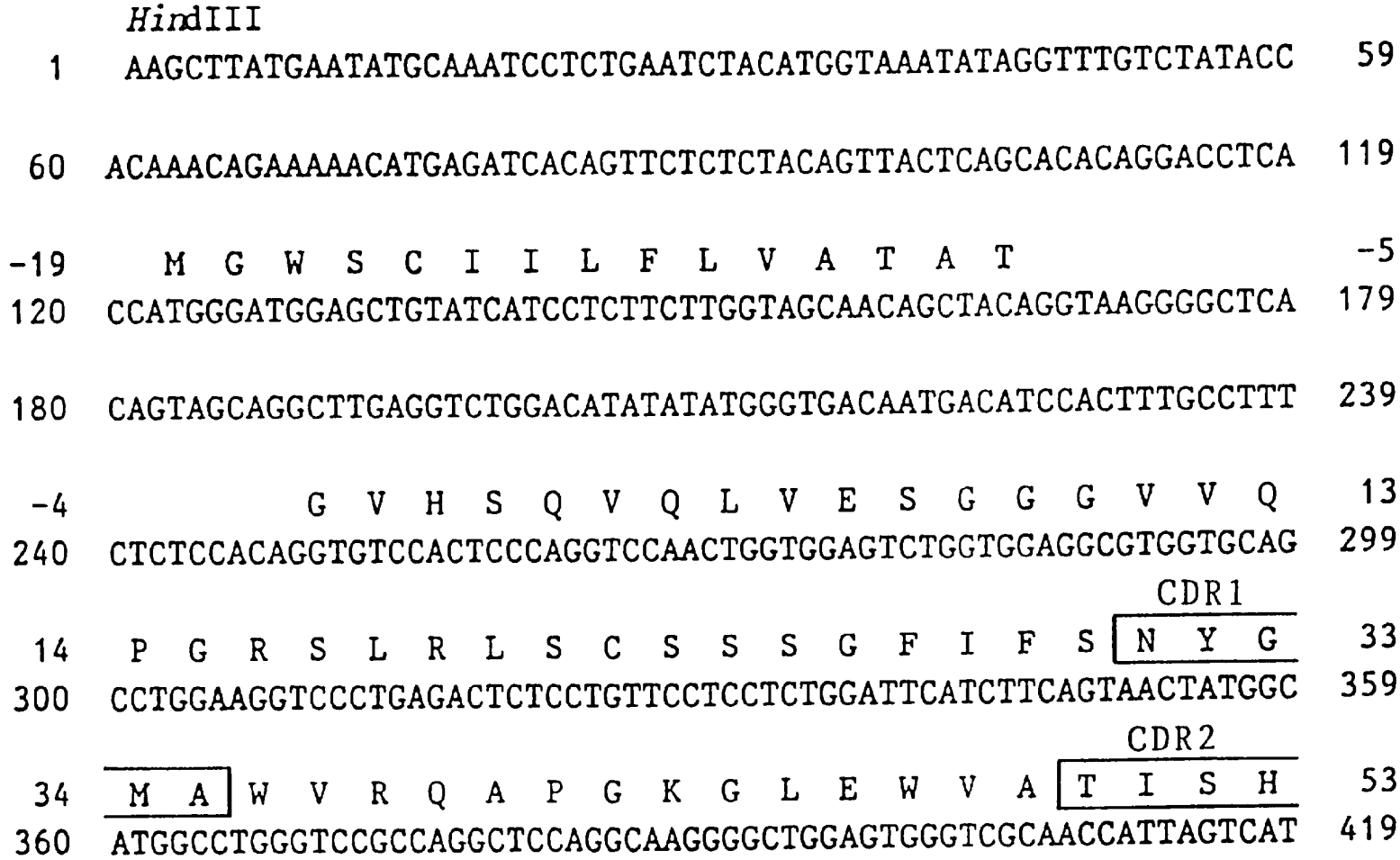
HindIII
1 AAGCTTATGAATATGCAAATCCTCTGAATCTACATGGTAAATATAGGTTTGTCTATACC 59
60 ACAAACAGAAAAACATGAGATCACAGTTCTCTCTACAGTTACTCAGCACACAGGACCTCA 119
-19 M G W S C I I L F L V A T A T -5
120 CCATGGGATGGAGCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTAAGGGGCTCA 179
180 CAGTAGCAGGCTTGAGGTCTGGACATATATATGGGTGACAATGACATCCACTTTGCCTTT 239
-4 G V H S Q V Q L V E S G G G V V Q 13
240 CTCTCCACAGGTGTCCACTCCCAGGTCCAACTGGTGGAGTCTGGTGGAGGCGTGGTGCAG 299
CDR1
14 P G R S L R L S C S S S G F I F S N Y G 33
300 CCTGGAAGGTCCCTGAGACTCTCCTGTTCCTCCTCTGGATTCATCTTCAGTAACTATGGC 359
CDR2
34 M A W V R Q A P G K G L E W V A T I S H 53
360 ATGGCCTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTCGCAACCATTAGTCAT 419

FIG.9 (contd.)

```
 54   D  G  S  D  T  Y  F  R  D  S  V  K  G  R  F  T  I  S  R  D   73
420   GATGGTAGTGACACTTACTTTCGAGACTCCGTGAAGGGCCGATTCACTATCTCCAGAGAT  479

 74   N  S  K  N  T  L  F  L  Q  M  D  S  L  R  P  E  D  T  G  V   93
480   AATAGCAAAAACACCCTATTCCTGCAAATGGACAGTCTGAGGCCCGAGGACACGGGCGTG  539

                              CDR 3
 94   Y  F  C  A  R  Q  G  T  I  A  G  I  R  H  W  G  Q  G  T  P   113
540   TATTTCTGTGCAAGACAAGGGACTATAGCAGGTATACGTCACTGGGGCCAAGGGACCCCC  599

114   V  T  V  S  S                                                118
600   GTCACCGTCTCCTCAGGTGAGTCCTTACAACCTCTCTCTTCTATTCAGCTTAAATAGATT  659

660   TTACTGCATTTGTTGGGGGGGAAATGTGTGTATCTGAATTTCAGGTCATGAAGGACTAGG  719

720   GACACCTTGGGAGTCAGAAAGGGTCATTGGGAGCCCGGGCTGATGCAGACAGACATCCTC  779

                                    BamHI
780   AGCTCCCAGACTTCATGGCCAGAGATTTATAGGGATCC                        817
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FIG. 10

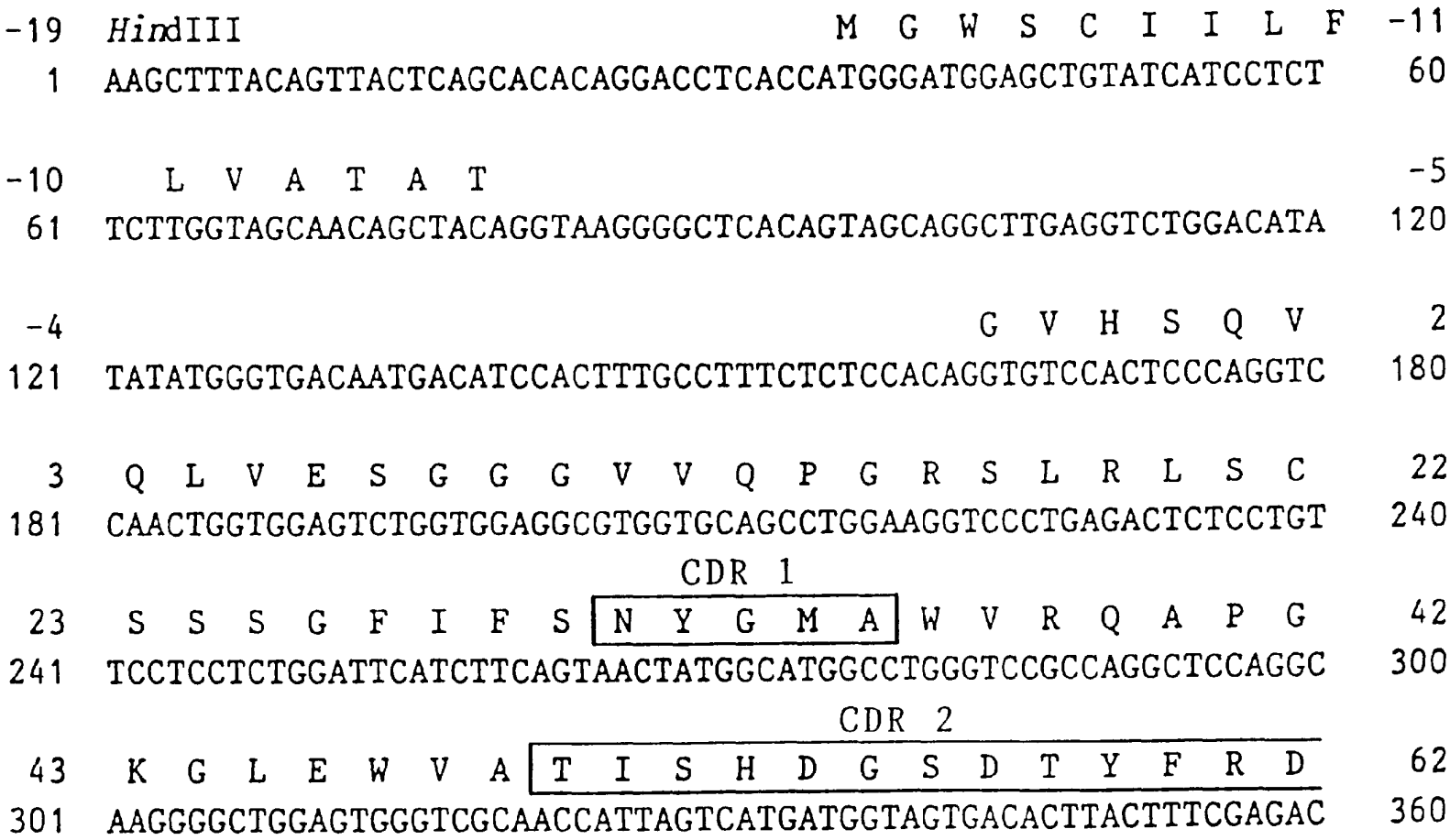
-19 HindIII M G W S C I I L F -11
1 AAGCTTTACAGTTACTCAGCACACAGGACCTCACCATGGGATGGAGCTGTATCATCCTCT 60
-10 L V A T A T -5
61 TCTTGGTAGCAACAGCTACAGGTAAGGGGCTCACAGTAGCAGGCTTGAGGTCTGGACATA 120
-4 G V H S Q V 2
121 TATATGGGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGGTC 180
3 Q L V E S G G G V V Q P G R S L R L S C 22
181 CAACTGGTGGAGTCTGGTGGAGGCGTGGTGCAGCCTGGAAGGTCCCTGAGACTCTCCTGT 240
CDR 1
23 S S S G F I F S N Y G M A W V R Q A P G 42
241 TCCTCCTCTGGATTCATCTTCAGTAACTATGGCATGGCCTGGGTCCGCCAGGCTCCAGGC 300
CDR 2
43 K G L E W V A T I S H D G S D T Y F R D 62
301 AAGGGGCTGGAGTGGGTCGCAACCATTAGTCATGATGGTAGTGACACTTACTTTCGAGAC 360

FIG. 10 (contd.)

```
 63  S V K G R F T I S R D N S K N T L F L Q  82
361  TCCGTGAAGGGCCGATTCACTATCTCCAGAGATAATAGCAAAAACACCCTATTCCTGCAA  420

                                        CDR 3
 83  M D S L R P E D T G V Y F C A R Q G T I  102
421  ATGGACAGTCTGAGGCCCGAGGACACGGGCGTGTATTTCTGTGCAAGACAAGGGACTATA  480

103  A G I R H W G Q G T P V T V S S  122
481  GCAGGTATACGTCACTGGGGCCAAGGGACCCCCGTCACCGTCTCCTCAGGTGAGTCCTTA  540

541  CAACCTCTCTCTTCTATTCAGCTTAAATAGATTTTACTGCATTTGTTGGGGGGGAAATGT  600

601  GTGTATCTGAATTTCAGGTCATGAAGGACTAGGGACACCTTGGGAGTCAGAAAGGGTCAT  660

661  TGGGAGCCCGGGCTGATGCAGACAGACATCCTCAGCTCCCAGACTTCATGGCCAGAGATT  720

          BamHI
721  TATAGGGATCC  731
```

FIG.11

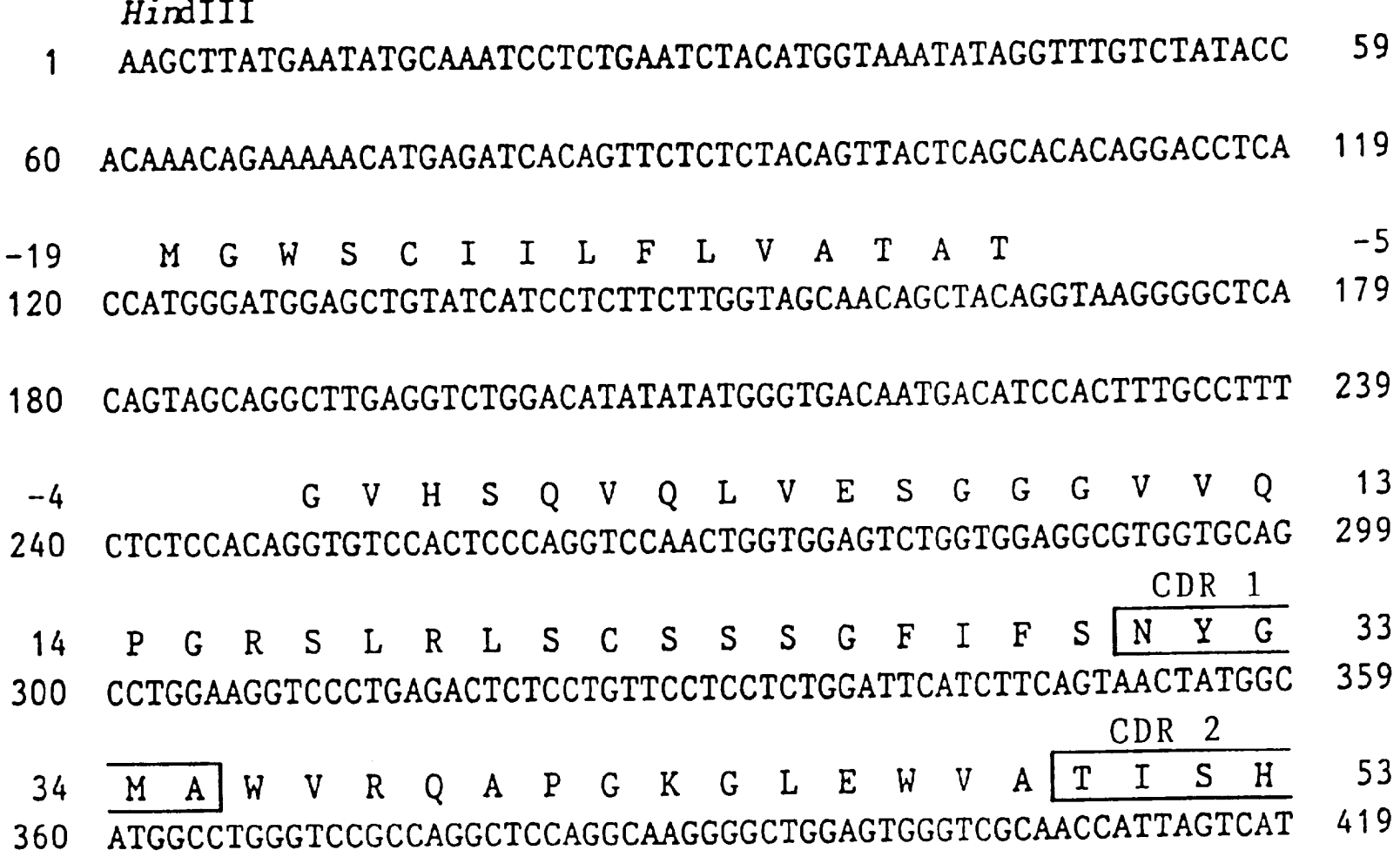

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FIG.11 (contd.)

```
 54  D G S D T Y F R D S V K G R F T I S R D   73
420  GATGGTAGTGACACTTACTTTCGAGACTCCGTGAAGGGCCGATTCACTATCTCCAGAGAT  479

 74  N S K N T L F L Q M D S L R P E D T G V   93
480  AATAGCAAAAACACCCTATTCCTGCAAATGGACAGTCTGAGGCCCGAGGACACGGGCGTG  539

                    CDR 3
 94  Y F C A R Q G T I A G I R H W G Q G T T  113
540  TATTTCTGTGCAAGACAAGGGACTATAGCAGGTATACGTCACTGGGGCCAAGGGACCACG  599

114  V T V S S                               118
600  GTCACCGTCTCCTCAGGTGAGTCCTTACAACCTCTCTCTTCTATTCAGCTTAAATAGATT  659

660  TTACTGCATTTGTTGGGGGGGAAATGTGTGTATCTGAATTTCAGGTCATGAAGGACTAGG  719

720  GACACCTTGGGAGTCAGAAAGGGTCATTGGGAGCCCGGGCTGATGCAGACAGACATCCTC  779

                          BamHI
780  AGCTCCCAGACTTCATGGCCAGAGATTTATAGGGATCC                        817
```

FIG. 12

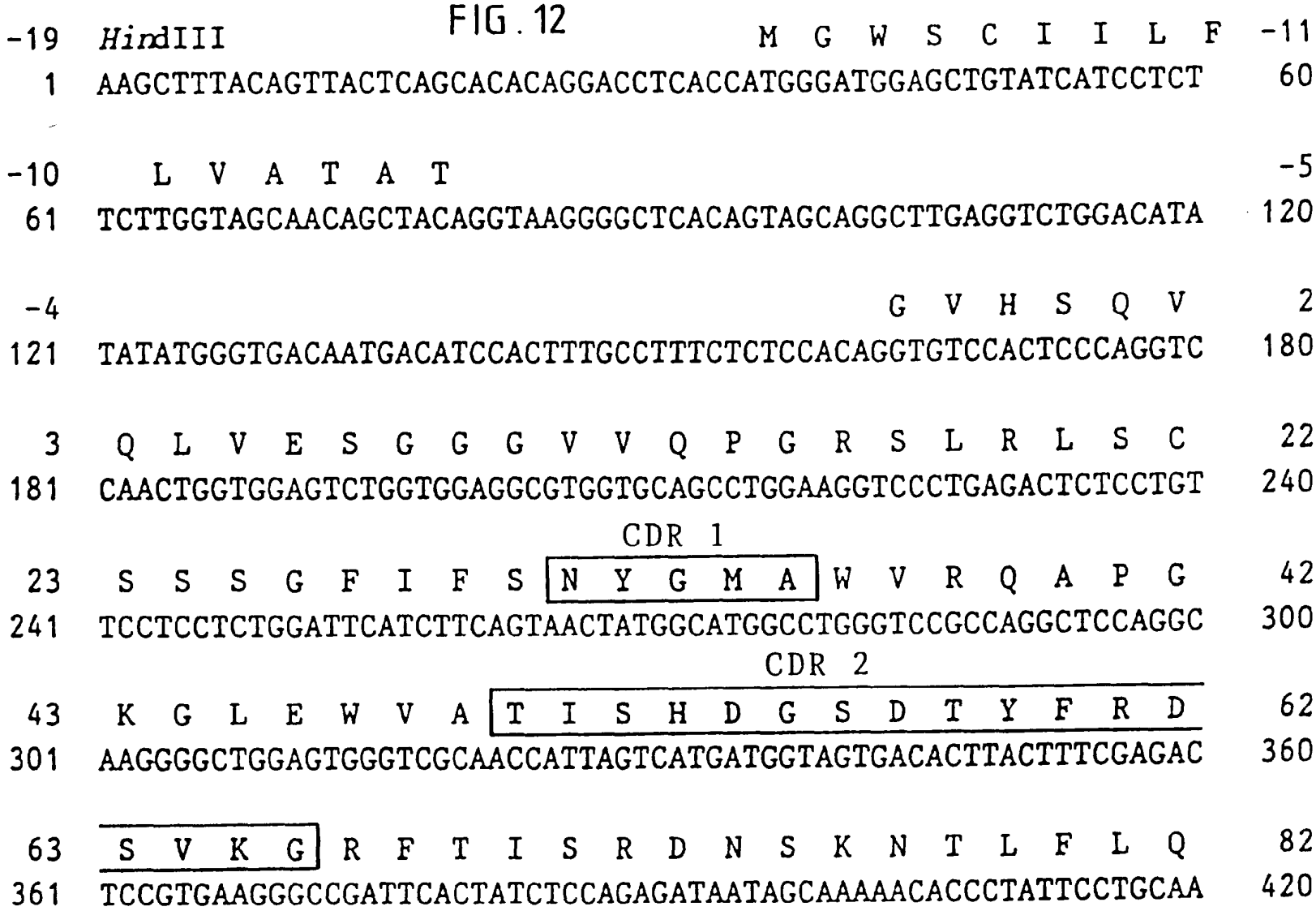

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FIG. 12 (contd.)

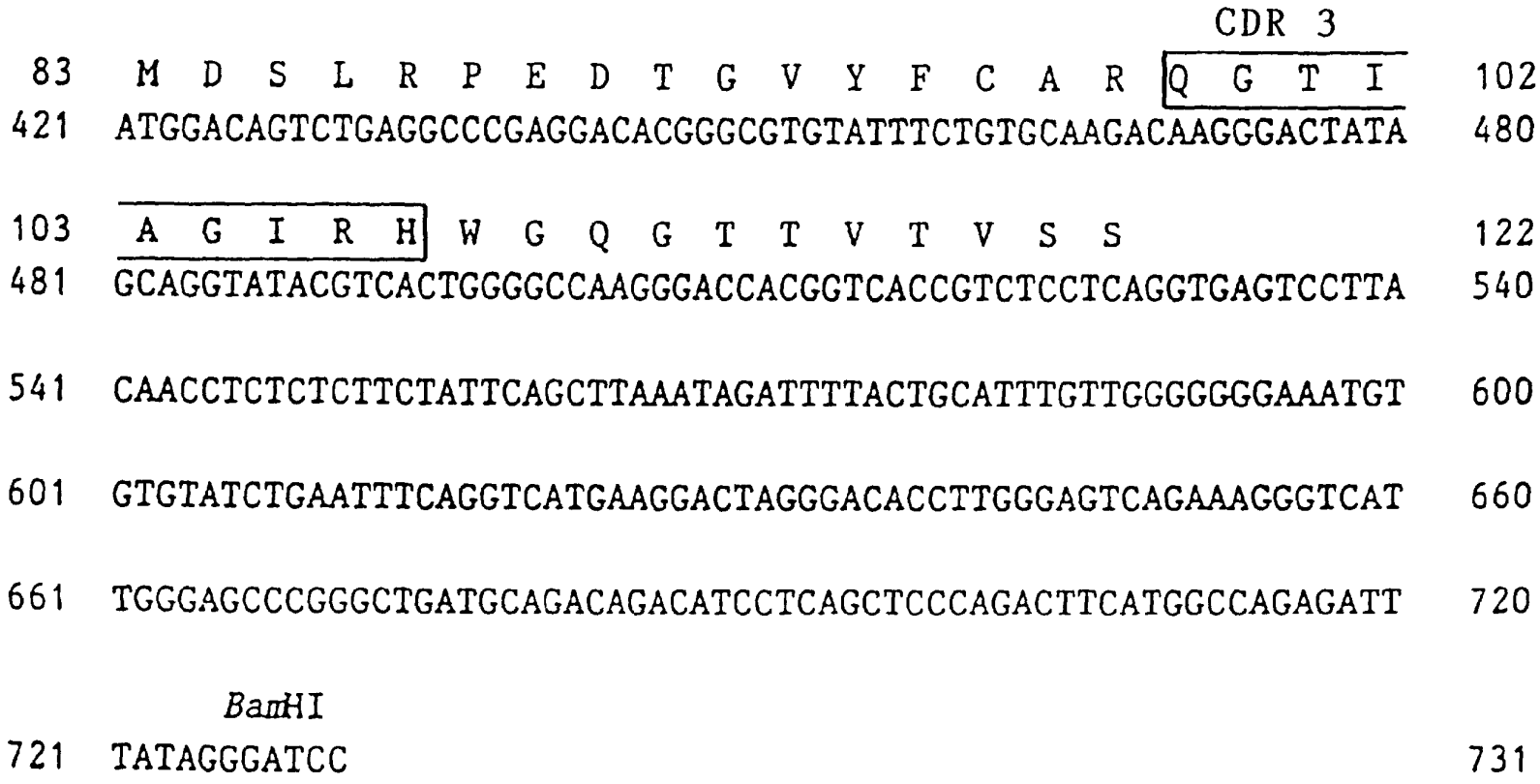

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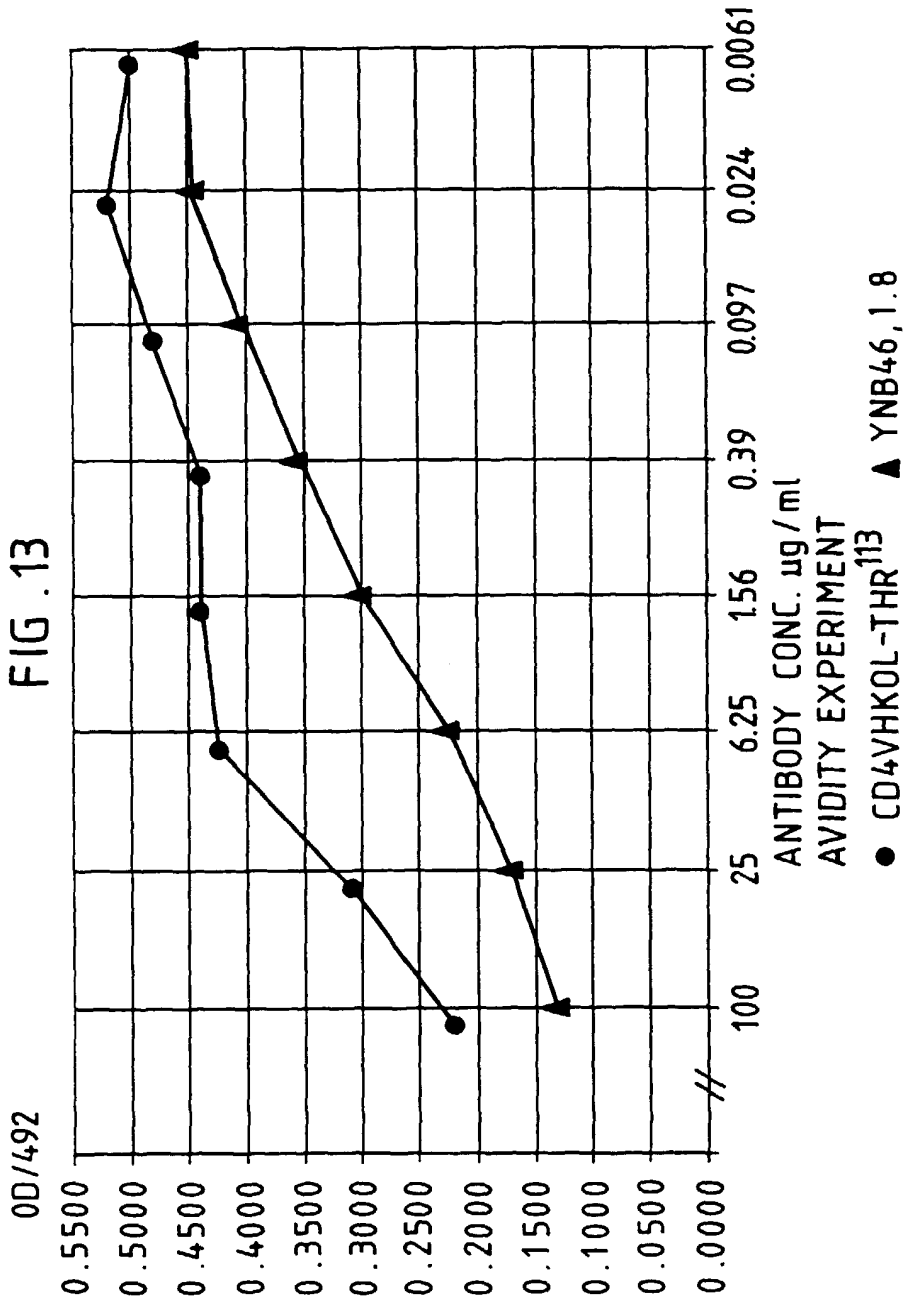
FIG .13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25
6.25
1.56
0.39
0.097
0.024
0.0061
ANTIBODY CONC. µg/ml
AVIDITY EXPERIMENT
● CO4VHKOL-THR113
▲ YNB46,1.8